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☐	L4	L3 not l2	57
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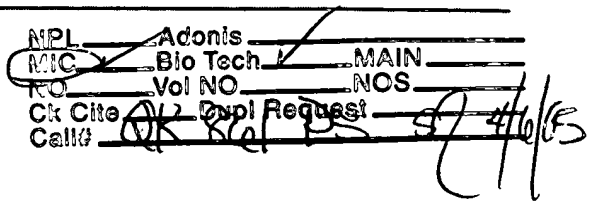

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STABILIZATION OF ISOLATED PHOTOSYSTEM II REACTION CENTER COMPLEX IN THE DARK AND IN THE LIGHT USING POLYETHYLENE GLYCOL AND AN %OXYGEN%-%SCRUBBING% SYSTEM
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Stabilization of Isolated Photosystem II Reaction Center Complex in the Dark and in the Light Using Polyethylene Glycol and an Oxygen-Scrubbing System[1]

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ABSTRACT

The photosystem II reaction center as isolated (O Nanba, K Satoh [1987] Proc Natl Acad Sci USA 84: 109–112) is quite dilute and very unstable. Precipitating the complex with polyethylene glycol and resuspending it in buffer without detergent concentrates the reaction center and greatly improves its stability at 4°C in the dark as judged by light-induced electron transport activity. Furthermore, a procedure was developed to minimize photodestruction of polyethylene-glycol-concentrated material at room temperature in the light. The ability to stabilize the photosystem II reaction center should facilitate future photophysical, biochemical, and structural studies of the complex.

The similarity between the primary structures of the L-M dimer of the bacterial reaction center and the D1-D2 proteins of PSII (19, 25) suggested an analogous structural and functional relationship between the reaction center of both types of organisms (7, 22). This suggestion was recently supported by the isolation of the D1-D2-Cyt *b*-559 complex from spinach (17) and, more recently, pea (1). This complex contains four to five chlorophylls, two Pheos,[5] one β-carotene, and one to two heme prosthetic groups per reaction center, but it lacks quinones and Mn (17). Nevertheless, photophysical studies demonstrated that the complex was the simplest PSII preparation yet isolated that still performs the following primary photochemical step (6):

$$P_{680} + \text{Pheo} \xrightarrow{h\nu} P_{680}^{+} + \text{Pheo}^{-} \qquad (1)$$

where P_{680} is the primary donor. The complex also catalyzes light-induced electron transport from 1,5-DPC to SiMo (5). These properties make the complex of considerable interest for further biochemical and photophysical studies; however, the complex is isolated at low concentration and is rather unstable (5, 21).

To address these problems, we have attempted to both concentrate and, more importantly, stabilize the complex by several methods. We found that precipitating the reaction center with PEG and resuspending it in buffer with or without small amounts of the nonionic detergent, lauryl maltoside, concentrates the reaction center material and greatly improves its stability, both in the dark and in the light. A previous paper (21) describes an earlier version of the PEG technique, and a preliminary report of the current work has appeared (16).

MATERIALS AND METHODS

Reaction center was isolated according to Nanba and Satoh (16) from PSII-enriched appressed spinach (*Spinacia oleracea* L.) membrane fragments (8) prepared by the method of Kuwabara and Murata (12). The membranes were stored at −80°C until use. Thirty mL of PSII membranes (1 mg Chl/mL) were solubilized in 4% Triton X-100, 50 mM Tris-HCl (pH 7.2) for 1 h at 4°C and centrifuged at 33,000*g* for 1 h (pelleting at ultrahigh *g* values reported in Ref. 17 is not necessary). The supernatant was loaded onto a 1.6 × 15 cm anion-exchange column containing Fractogel TSK-DEAE 650S now sold by Supelco, Bellefonte, PA, under the trade name TSK-GEL DEAE-Toyopearl 650S. The column was washed with approximately 4 L of 50 mM Tris-HCl (pH 7.2), 0.05% Triton (buffer A) containing 30 mM NaCl overnight until the absorbance (1-cm pathlength) at 670 nm of the eluant was below 0.01. The reaction center material was then eluted with a 300 mL 30 to 200 mM NaCl gradient in buffer A. The green reaction center fraction, hereafter termed control reaction center, was concentrated by one of the following three techniques.

Concentration Ion-Exchange Column

Control reaction center was diluted with an equal volume of buffer A and loaded onto a second Fractogel column (1.1 × 4 cm). The column was washed with buffer A containing 30 mM NaCl and the reaction center eluted with 150 mM NaCl.

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[4] A division of the Midwest Research Institute operated for the U.S. Department of Energy under contract No. DE-AC02-83CH-10093.

[5] Abbreviations: Pheo, pheophytin; DPC, diphenylcarbazide; SiMo, silicomolybdate; LDS, lithium dodecyl sulfate; IE, ion exchange; UC, ultracentrifuge.

Ultracentrifugation of Highly Diluted Material

Control reaction center was diluted 1:7 with 50 mM Tris-HCl (pH 7.2) (buffer B) and centrifuged at 309,000*g* for 3 h to pellet aggregated reaction center (this procedure was suggested to us by Dr. M. Ikeuchi, The Institute of Physical and Chemical Research, Wako-shi, Japan). The pellet was resuspended in the same buffer with or without added detergent.

PEG Precipitation

To control reaction center, 0.325 g of PEG (Sigma; mol wt = 3,350) per mL was added slowly and dispersed carefully with a small paintbrush. After 90 min incubation, the suspension was centrifuged at 31,300*g* for 15 min (see also Ref. 21). The pellet was resuspended in buffer B with detergent as noted in the tables, and centrifuged again at 1,100*g* for 90 s to pellet mostly colorless material containing PEG aggregates. The exact conditions of the last centrifugation may have to be adjusted if green material appears in the pellet. All preparative procedures were done at 4°C in the dark, LDS-PAGE was performed as in Seibert *et al.* (21), and absorption spectra obtained with an HP 8450A diode array spectrophotometer.

Photochemical competence of the reaction center was assayed by photoinduced electron transport from DPC to SiMo (1) at 4°C, monitored by observing absorbance increases at 600 nm, with a Cary 17D spectrophotometer. Reaction center was diluted to 0.7 μg Chl/mL (final concentration) in 60 mM Tris-HCl (pH 8.5), 0.025% Triton. After incubation for 2 min, DPC (Sigma) was added to 43 μg/mL and SiMo (Pfaltz and Bauer, Waterbury, CT) to 200 μg/mL, and the sample was illuminated from the top using a slide projector. Actinic light was passed through a Schott RG 630 cutoff filter and a hot mirror filter (Melles Griot No. 03MHG007). The light intensity was about 800 W/m^2 at the sample. The photomultiplier was shielded with a narrow band 600 nm interference filter (Melles Griot No. 03FIV045). Activity was calculated using an extinction coefficient of 4.8 mM^{-1} · cm^{-1} (5) for SiMo. The photochemical activity of this material, representing the 100% values in the tables, ranged from 1350 to 2250 μmol SiMo reduced · mg Chl^{-1} · h^{-1}, depending on the preparation. This is 5 to 11 times that previously reported for

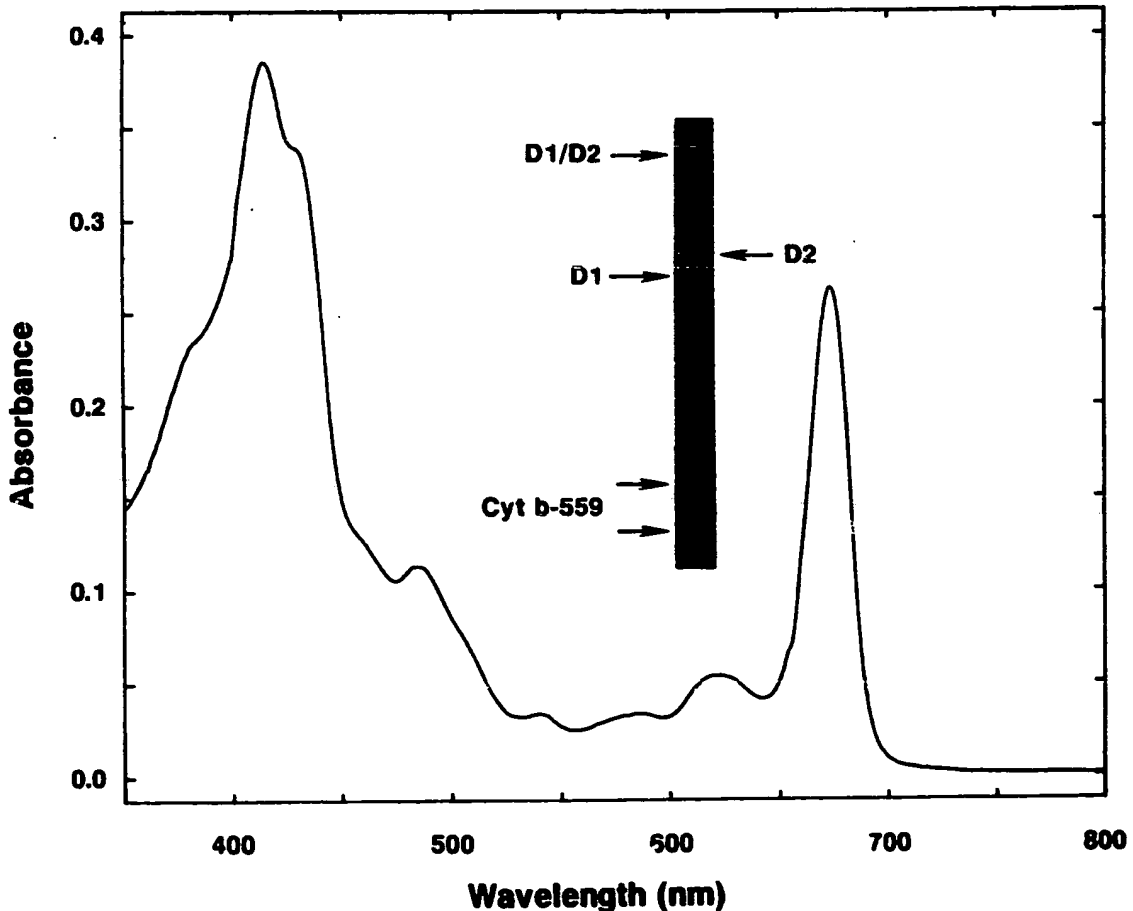

Figure 1. Absorption spectrum and LDS-PAGE profiles of PEG-concentrated PSII reaction center isolated from spinach. The spectrum was obtained after dilution in 50 mM Tris-HCl (pH 7.2). The small shoulder at about 655 nm is an instrument artifact. The LDS-PAGE (6 M urea) was run at 4°C in a 12.5% gel as in Ref. 21. Samples were denatured at room temperature for 30 min in a solution containing 50 mM Tris-HCl (pH 7.2), 2% (w/v) LDS, 2 M urea, 40 mM dithiothreitol, and 20% (w/v) sucrose. The gel was stained with Coomassie brilliant blue. D1, D2, D1/D2, and Cyt *b*-559 are discussed in the text.

Table I. *Photochemical Activity of PSII Reaction Center Complex during Aging in the Dark at 4°C*

Control reaction center material (off the first column), reaction center concentrated by a second IE column, and reaction center concentrated by UC are compared. Control and IE samples contained 0.05% Triton X-100. The UC samples were resuspended in Tris buffer with or without lauryl maltoside as indicated in parentheses. Activities are expressed as a percentage of the initial DPC to SiMo activity at zero time for each sample (see "Materials and Methods").

Aging Time	Sample	Activity
		%
6 h	Control RC[a]	79
	IE[b]	76
1 d	Control RC	56
	IE	57
	UC (no detergent)[c]	77
	UC (0.1% lauryl maltoside)[c]	79
4 d	Control RC	38
	IE	39
	UC (no detergent)	56
	UC (0.1% lauryl maltoside)	46

[a] Chl = 4.95 μg/mL during aging. [b] Chl = 26 μg/mL during aging. [c] Chl = 26.5 μg/mL during aging.

Table II. *Photochemical Activity of PEG-Concentrated PSII Reaction Center Samples Resuspended in Different Detergents and Detergent Concentrations and Then Aged in the Dark at 4°C*

DPC to SiMo activities are expressed as a percentage of the initial activity at zero time for each sample. Samples were aged at 43 μg Chl/mL.

Aging Time	PEG-Concentrated Sample	Activity
		%
1 d	No detergent	83[a]
	0.1% lauryl maltoside	88
	0.4% lauryl maltoside	81
	0.05% Triton X-100	66
4 d	No detergent	80[a]
	0.1% lauryl maltoside	82
	0.4% lauryl maltoside	75
	0.05% Triton X-100	53
8 d	No detergent	81[a]
	0.1% lauryl maltoside	76
	0.4% lauryl maltoside	66
	0.05% Triton X-100	42

[a] The PEG-concentrated samples resuspended in the absence of detergent exhibit stable activity from d 1 to d 8 under these conditions. Thus, the scatter in the data (80–83%) gives an estimation of the experimental error in this and the other tables.

isolated PSII reaction center complex (5) assuming that SiMo is a four-electron acceptor at physiological pH. Triton was required in the assay buffer to eliminate reaction center aggregates, and its addition resulted in improved electron transport rates.

Anaerobic conditions were created by adding at final concentration 20 mM glucose, 0.039 mg/mL catalase (Sigma C-100, EC 1.11.1.6), and 0.1 mg/mL glucose oxidase (Sigma G-6125, EC 1.1.3.4, type II) in that order to the samples under an argon atmosphere.

Table III. *Stability of Control PSII Reaction Center and of PEG-Concentrated PSII Reaction Center Resuspended in Tris Buffer without Detergent in the Dark and Light at 22°C*

Samples were kept in the dark or illuminated (8.5 W/m² from a cool-white fluorescent tube) for 45 min. Activities are expressed as a percentage of the initial DPC to SiMo activity at zero time for each sample. The Chl concentration of the samples during aging was 6.1 μg/mL.

Sample	Treatment	Activity
		%
Control RC	Aerobic in the dark	44
	Aerobic in the light	16
	Anaerobic in the light	29
PEG-concentrated	Aerobic in the dark	92
	Aerobic in the light	46
	Anaerobic in the light	93

RESULTS AND DISCUSSION

The absorption spectrum of PEG-precipitated PSII reaction center (Fig. 1) shows major peaks and shoulders at 674, 622, 540, 484, 434, and 414 nm in the visible region. Figure 1 also shows a 6 M urea LDS-PAGE run of the same preparation. Four major polypeptides identified previously by immunological techniques (20) are visible at about 34 (D2), 31 (D1), and 9 and 5 (Cyt *b*-559) kD. The band at about 60 kD corresponds to dimers of D1/D2. Both the absorbance spectrum and the electrophoresis patterns are quite similar to those published previously (16).

Tables I and II show light-induced electron transport activity losses during aging of reaction center material subjected to the different concentration procedures and are representative single experiments. From these tables we can deduce the factors involved in stabilization of reaction center activity. Concentration of reaction center by IE chromatography tested whether simply increasing the protein to detergent ratio would improve stability. The data in Table I show that it did not. Another approach examined was to remove excess Triton or to replace excess Triton with a milder detergent, such as lauryl maltoside. Ultracentrifugation of highly diluted reaction center material and subsequent resuspension (see "Materials and Methods") allowed this removal or replacement. Comparison of the UC reaction center data with those of control and ion-exchange concentrated reaction center in Table I shows that replacing Triton with lauryl maltoside improved stability. However, equivalent or even slightly greater stability was achieved by removing excess Triton and resuspending the material in Tris buffer without detergent. The same effects of Triton removal or replacement are seen in the PEG-precipitated samples of Table II. A comparison of Tables I and II shows that the PEG-precipitated samples were more stable than the ultracentrifuged ones. This indicates that PEG has a stabilizing effect independent of detergent removal or replacement. This is confirmed by comparing control and ion-exchange-concentrated sample (both of which contain 0.05% Triton) data with the data for PEG-concentrated sample resuspended in 0.05% Triton. The stabilizing effects of PEG and those of detergent removal combined to give our most stable preparations. PEG-precipitated reaction center resus-

pended in Tris buffer without detergent or with small amounts (0.1%) of lauryl maltoside (Table II).

It should be noted that comparison of the data in Tables I and II could be affected by the fact that samples were aged at different Chl concentrations. However, the control and ion-exchange-concentrating column data (Table I) indicate that reaction center concentration is not very important for stability during the aging period. We also found that storage of PEG-precipitated PSII reaction center samples, resuspended without detergent at concentrations from 8 to 73 μg Chl/mL, had no effect on stability under the conditions of Table II (data not shown).

Precipitation of PSII reaction center with PEG decreased the photochemical activity of the preparation by about 10% compared to control samples. However, this small apparent loss in activity may be an artifact of the assay caused by some PEG-induced aggregates of reaction center remaining during the assay. As mentioned earlier, the assay buffer contained 0.025% Triton which was necessary to obtain maximum electron transport rates. We assume that Triton breaks down the reaction center aggregates, favoring the interaction of the electron donor (DPC) and acceptor (SiMo) with the reaction center. Self-aggregation of proteins induced by PEG has been reported in the literature (13, 18). The other methods of concentration used in this study, ion-exchange chromatography and ultracentrifugation of diluted material, also caused little loss of photochemical activity. In any case, we emphasize that the small losses due to concentration procedure are distinct from the aging losses noted in the tables.

The stabilizing effect of replacing a harsh detergent by a mild detergent reported here has also been demonstrated with bacterial reaction centers (11) and O_2-evolving core complex from cyanobacteria (4). Also, the present use of PEG adds to the list of previous applications of this compound to precipitate proteins (10) and to stabilize enzyme activity (9, 15).

Control reaction center was unstable in the dark and even more so in the light at 22°C (Table III, see also Ref. 5). The experiment in Table III also shows that PEG precipitation of reaction center improved stability both in the dark and in the light at this temperature. However, exposure to anaerobic conditions during illumination further improved the protection of reaction center against photodestruction, virtually eliminating it in PEG-precipitated samples under these conditions. Prevention of photodestruction under anaerobic conditions strongly suggests that some photoproduct(s) of oxygen mediates the destruction. Singlet oxygen and/or superoxide radical are probable candidates. It is well known that porphyrins (2, 14, 23) are very efficient photogenerators of these destructive agents. Surprisingly, PSII reaction center seems to be more sensitive to photodamage effects than bacterial reaction center (3), despite the presence of the same amount of carotenoid in both reaction center species. Current studies in our laboratory are aimed at understanding in more detail the mechanism of the observed photodestruction of PSII reaction center. Finally, the development of effective means to stabilize the PSII reaction center reported in this paper should facilitate future photophysical, biochemical, and structural studies of the complex. For example, PEG-precipitated PSII reaction center, illuminated under anaerobic conditions as described in this paper, was stable enough to allow prolonged exposure to 500 femtosecond laser flashes necessary to measure the risetime of P^+_{680} at 4°C (24).

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12413845 PMID: 9726283

Role of catalase in in vitro acetaldehyde formation by human colonic contents.

Tillonen J; Kaihovaara P; Jousimies-Somer H; Heine R; Salaspuro M

Research Unit of Alcohol Diseases, University Central Hospital of Helsinki, Finland.

Alcoholism, clinical and experimental research (UNITED STATES) Aug 1998 , 22 (5) p1113-9, ISSN 0145-6008 Journal Code: 7707242

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Ingested ethanol is transported to the colon via blood circulation, and intracolonic ethanol levels are equal to those of the blood ethanol levels. In the large intestine, ethanol is oxidized by colonic **bacteria** , and this can lead to extraordinarily high acetaldehyde levels that might be responsible, in part, for ethanol-associated carcinogenicity and gastrointestinal symptoms. It is believed that **bacterial** acetaldehyde formation is mediated via microbial alcohol dehydrogenases (ADHs). However, almost all cytochrome-containing aerobic and facultative **anaerobic bacteria** possess catalase activity, and catalase can, in the presence of hydrogen peroxide (H2O2), use several alcohols (e.g., ethanol) as substrates and convert them to their corresponding aldehydes. In this study we demonstrate acetaldehyde production from ethanol in vitro by colonic contents in a reaction catalyzed by both **bacterial** ADH and catalase. The amount of acetaldehyde produced by the human colonic contents was proportional to the ethanol concentration, the amount of colonic contents, and the length of incubation time, even in the absence of added nicotinamide adenine dinucleotide or H2O2. The catalase inhibitors sodium **azide** and 3-amino-1,2,4-triazole (3-AT) markedly reduced the amount of acetaldehyde produced from 22 mM ethanol in a concentration dependent manner compared with the control samples (0.1 mM sodium **azide** to 73% and 10 mM 3-AT to 67% of control). H2O2 generating system [beta-D(+)-glucose + glucose **oxidase**] and nicotinamide adenine dinucleotide induced acetaldehyde formation up to 6- and 5-fold, respectively, and together these increased acetaldehyde formation up to 11-fold. The mean supernatant catalase activity was 0.53+/-0.1 micromol/min/mg protein after the addition of 10 mM H2O2, and there was a significant ($p < 0.05$) correlation between catalase activity and acetaldehyde production after the addition of the hydrogen peroxide generating system. Our results demonstrate that colonic contents possess catalase activity, which probably is of **bacterial** origin, and indicate that in addition to ADH, part of the acetaldehyde produced in the large intestine during ethanol metabolism can be catalase dependent.

Tags: Female; Male; Research Support, Non-U.S. Gov't

Descriptors: *Acetaldehyde--pharmacokinetics--PK; * **Bacteria** --enzymology --EN; *Catalase--physiology--PH; *Colon--microbiology--MI; *Ethanol --pharmacokinetics--PK; *Gastrointestinal Contents--microbiology--MI; Adult ; Aged; Alcohol Dehydrogenase--physiology--PH; Humans; Hydrogen Peroxide --metabolism--ME; Middle Aged

CAS Registry No.: 64-17-5 (Ethanol); 75-07-0 (Acetaldehyde); 7722-84-1 (Hydrogen Peroxide)

Enzyme No.: EC 1.1.1.1 (Alcohol Dehydrogenase); EC 1.11.1.6 (Catalase)

Record Date Created: 19981215

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10191130 PMID: 8503894

Rubredoxin oxidase , **a new flavo-hemo-protein, is the site of oxygen reduction to water by the "strict** anaerobe " **Desulfovibrio gigas.**

Chen L; Liu M Y; LeGall J; Fareleira P; Santos H; Xavier A V

Department of Biochemistry, University of Georgia, Athens 30602.

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A rubredoxin-oxygen oxidoreductase, a homodimer with a molecular weight of 43 kDa per monomer, was found to be a component of an electron transfer chain that couples the reduction of oxygen to water with NADH oxidation. This FAD-containing protein appears to contain a new type of heme group. The electron transfer chain is not inhibited by cyanide and **azide** . In contrast, CO decreases NADH oxidation rate and also induces release of the prosthetic groups from the native terminal reductase.

Tags: Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Desulfovibrio--metabolism--ME; *Oxidoreductases--metabolism --ME; *Oxygen--metabolism--ME; *Water--metabolism--ME; Chromatography, Gel; Chromatography, Paper; Desulfovibrio--enzymology--EN; Electron Transport; Electrophoresis, Polyacrylamide Gel; NAD--metabolism--ME; Oxidation-Reducti on; Oxidoreductases--isolation and purification--IP; Spectrophotometry, Ultraviolet

CAS Registry No.: 53-84-9 (NAD); 7732-18-5 (Water); 7782-44-7 (Oxygen)

Enzyme No.: EC 1. (Oxidoreductases); EC 1.9.3.- (rubredoxin-oxygen oxidoreductase)

Record Date Created: 19930701

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04508044 PMID: 124899

Studies on the energy metabolism during anaerobic **fermentation of glucose by baker's yeast.**

Hoogerheide J C

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Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

As a result of the intimate association of ADP phosphorylation with alcoholic fermentation, resulting in the synthesis of 2 mole ATP per mole glucose fermented, it may be calculated that a minimum of 672 mucal heat development may be expected for every mm-3 CO2 developed during alcoholic fermentation. When all ATP produced would be fully de-phosphorylated to ADP + Pi (e.g. by ATP-ase activity) a maximum heat development of 1200 mucal per mm-3 CO2 could be expected. Using the LKB-Flow-Microcalorimeter for measurement of heat development and at the same time the Warburg technique for measuring CO2 development during **anaerobic** glucose fermentation of a baker's yeast suspension, the heat development per mm-3 CO2 produced was calculated over a fermentation period of 90 min. Maintenance of strict **anaerobic** conditions in the Flow-Microcalorimeter vessel was complicated by diffusion of traces of oxygen via the Teflon transport lines, resulting in excessive heat development values, not representative for the alcoholic fermentation. This problem could be circumvented by removal of traces of oxygen by means of addition of the enzyme glucose- **oxidase** . Poisoning the respiratory enzyme system of the yeast by addition of KCN or **azide** , or using respiratory-deficient mutants of the yeast also resulted in heat development values, inherent with alcoholic fermentation. The values obtained were very close to the minimum of 672 mucal per mm-3 CO2, at least during the initial phases of fermentation, indicating that ADP regeneration from ATP, essential for maintaining the high fermentation rate, is not primarily the result of ATP-ase activity, but must be due to participation of ATP in energy-requiring synthetic reactions.

Descriptors: ***Anaerobiosis** ; *Fermentation; *Glucose--metabolism--ME; *Metabolism; *Saccharomyces cerevisiae--metabolism--ME; Adenosine Diphosphate--metabolism--ME; Adenosine Triphosphate--biosynthesis--BI; Adenosinetriphosphatase; **Azides** --pharmacology--PD; Calorimetry; Carbon Dioxide--biosynthesis--BI; Cyanides--pharmacology--PD; Energy Metabolism; Ethanol--biosynthesis--BI; Heat; Kinetics; Mutation; Potassium

CAS Registry No.: 0 (Azides); 0 (Cyanides); 124-38-9 (Carbon Dioxide); 50-99-7 (Glucose); 56-65-5 (Adenosine Triphosphate); 58-64-0 (Adenosine Diphosphate); 64-17-5 (Ethanol); 7440-09-7 (Potassium)

Enzyme No.: EC 3.6.1.3 (Adenosinetriphosphatase)

Record Date Created: 19751007

Record Date Completed: 19751007

Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The periplasmic nitrate reductase of Thiosphaera pantotropha has been purified from a mutant strain (M-6) that overproduces the enzyme activity
under *** anaerobic *** growth conditions. The enzyme is a complex of a
93-kDa polypeptide and a 16-kDa nitrate-oxidizable cytochrome c552. The
complex contains molybdenum; a fluorescent compound with spectral features
of a pterin derivative can be extracted. In contrast to the dissimilatory
membrane-bound nitrate reductases, the periplasmic nitrate reductase shows high specificity for nitrate as a substrate and is insensitive to
inhibition by *** azide *** . The 93-kDa subunit exhibits immunological
cross-reactivity with the catalytic subunit of Rhodobacter capsulatus
N22DNAR+ periplasmic nitrate reductase. Mass spectrometric comparisons of
holo-cytochrome c552 and apo-cytochrome c552 demonstrated that the
polypeptide bound two haem groups. *** Mediated *** redox potentiometry of
the cytochrome indicated that the haem groups have reduction potentials (pH
= 7.0) of approximately -15 mV and + 80 mV. The functional significance of
these potentials is discussed in relation to the proposed physiological
role of the enzyme as a redox valve.
*** Tags: Research Support, Non-U.S. Gov't***
Descriptors: *Nitrate Reductases--isolation and purification--IP; *Paracoccus denitrificans--enzymology--EN; Cell Membrane--enzymology--EN; Heme--chemistry--CH; Hydrogen-Ion Concentration; Membrane Potentials; Molecular Weight; Mutation; Nitrate Reductases--chemistry--CH; Nitrate Reductases--metabolism--ME; Oxidation-Reduction; Paracoccus denitrificans --genetics--GE; Spectrum Analysis, Mass; Substrate Specificity
*** Molecular Sequence Databank No.: GENBANK/Z36773***
*** CAS Registry No.: 14875-96-8 (Heme)***
*** Enzyme No.: EC 1.- (Nitrate Reductases); EC 1.7.99.4 (nitrate*** reductase)
Record Date Created: 19940401
Record Date Completed: 19940401

19/9/8
DIALOG(R)File 155:MEDLINE(R)
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10301153 PMID: 1285025
[Efficiency of inhibitors (phenylethanol, nalidixic acid, sodium azide)in the isolation of strictly anaerobic bacteria from a polymicrobial specimen]
*** Efficacite des inhibiteurs (P.E.A, Ac Nal, Az de Na) dans l'isolement des***
bacteries anaerobies strictes, a partir d'un prelevement polymicrobien.
Merad A S; Ghemati M
*** Laboratoire des Anaerobies, Institut Pasteur d'Algerie, Alger.***
*** Archives. Institut Pasteur d'Algerie (ALGERIA) 1992, 58 p161-8,***
ISSN 0020-2460 Journal Code: 0373031
Publishing Model Print
Document type: Journal Article ; English Abstract
Languages: FRENCH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The efficacy of inhibitors (PEA, Ac Nal, Az de Na) in the isolation

```
Set     Items   Description
S1        109   (MEMBRAN? (2N) FRAGMENT?) (100N) (AZIDE? OR NAN3)
S2         70   RD (unique items)
S3          5   S2 AND (ANAEROB? OR MICROAERO?)
? t s3/9/1-2

 3/9/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
(c) 2005 BIOSIS. All rts. reserv.

0006819528   BIOSIS NO.: 198988134643
DYNAMICS OF CHANGES IN THE ULTRASTRUCTURE OF ESCHERICHIA-COLI MEMBRANES
  DURING PLASMOLYSIS
AUTHOR: TARAKHOVSKII YU S (Reprint); MANUVAKHOVA M SH; GONGADZE G M
AUTHOR ADDRESS: INST BIOL PHYS, ACAD SCI USSR, PUSHCHINO, USSR**USSR
JOURNAL: Biologicheskie Membrany (Moscow)  6 (5): p530-540 1989
ISSN: 0233-4755
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: A detailed analysis of the ultrastructural changes in the E. coli
  membranes exposed to 20% sucrose has been performed over the time range 3
  s - 30 min. The most informative methods proved to be freeze-fracture
  with ultrarapid freezing and chemical fixation using a succession of
  glutaraldehyde, osmium tetroxide, tannin, and again osmium tetroxide. The
  traditional fixation with glutaraldehyde and osmium tetroxide failed to
  reveal a number of details. Plasmolysis was shown to be a multistage
  process. Within first 3 s blebbing of the outer membrane occurs and small
  vesicles are released into external medium. In next 10 min the cytoplasm
  shrinks and the periplasmic space expands. This is accompanied with
  numerous invaginations on the inner membrane and appearance of vesicles
  in the cytoplasm. The adhesion sites of the inner and outer membranes are
  observed in periplasmic space. After 10 min the adhesion sites begin to
  disappear with the concomitant segregation of the inner %membrane%
  %fragments% in the periplasm. At 30th min, cytoplasmic vesicles form a
  tight contact with the inner membrane that is followed by their
  exocytosis into periplasmic space. This process is not inhibited by
  merthiolate or %azide%.

DESCRIPTORS: INNER MEMBRANE FRAGMENTATION PERIPLASM CYTOPLASMIC VESICLE
FORMATION
DESCRIPTORS:
  MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Membranes--Cell
    Biology; Physiology
  BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively %Anaerobic%
    Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
  COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms
CONCEPT CODES:
  10506 Biophysics - Molecular properties and macromolecules
  10508 Biophysics - Membrane phenomena
  31000 Physiology and biochemistry of bacteria
  32300 Microbiological ultrastructure
BIOSYSTEMATIC CODES:
  06702 Enterobacteriaceae


 3/9/2     (Item 2 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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0006615710   BIOSIS NO.: 198987063601
CATALYTIC PROPERTIES OF THE MEMBRANE-BOUND ATPASE OF THE %ANAEROBIC%
  BACTERIUM LACTOBACILLUS-CASEI
```

AUTHOR: MIL'GROM YA M (Reprint); MUNTYAN M S; SKULACHEV V P
AUTHOR ADDRESS: AN BELOZERSKII INTERFAC PROBL RES LAB MOL BIOL BIOORG CHEM, MV LOMONOSOV MOSC STATE UNIV, MOSCOW, USSR**USSR
JOURNAL: Biologicheskie Membrany (Moscow) 5 (6): p565-572 1988
ISSN: 0233-4755
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

ABSTRACT: %Membrane% %fragments% of Lactobacillus casei possess Mg2+-stimulated DCCD-sensitive ATPase activity (0.3-0.5 μmol/min per mg of protein) with Km for ATP about 1 mM. Mg2+-stimulated ATPase activity of membranes is maximal at pH 6.0-6.2 and decreases sharply when pH rises to 6.7. Mg2+-ATPase activity of membranes is stimulated by sulfite and octylglucoside. In the presence of ATP-regenerating system, ATPase activity of membranes decreases in the course of ATP hydrolysis. This decrease is prevented by sulfite. %Azide% has no effect on the initial rate of ATP hydrolysis but enhances markedly the decrease of enzyme activity during ATP hydrolysis. Half-maximal inhibition of Mg2+-stimulated ATPase activity is caused by 15 μM axide. The inhibitory action of azide is reversed by sulfite. It follows from the results presented that there is an ATPase of F0F1-type in the membranes of L. casei.

REGISTRY NUMBERS: 9000-83-3: ATPASE; 14265-45-3: SULFITE; 14343-69-2: AZIDE
DESCRIPTORS: SULFITE AZIDE
DESCRIPTORS:
  MAJOR CONCEPTS: Bioenergetics--Biochemistry and Molecular Biophysics; Enzymology--Biochemistry and Molecular Biophysics; Membranes--Cell Biology; Physiology
  BIOSYSTEMATIC NAMES: Regular Nonsporing Gram-Positive Rods--Eubacteria, Bacteria, Microorganisms
  COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms
  CHEMICALS & BIOCHEMICALS: ATPASE; SULFITE; AZIDE
CONCEPT CODES:
  10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
  10064 Biochemistry studies - Proteins, peptides and amino acids
  10508 Biophysics - Membrane phenomena
  10510 Biophysics - Bioenergetics: electron transport and oxidative phosphorylation
  10808 Enzymes - Physiological studies
  31000 Physiology and biochemistry of bacteria
BIOSYSTEMATIC CODES:
  07830 Regular Nonsporing Gram-Positive Rods
? t s3/3/3-5

3/3/3 (Item 1 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

01011698
A MEDIUM COMPOSITION, METHOD AND DEVICE FOR SELECTIVELY ENHANCING THE ISOLATION OF %ANAEROBIC% MICROORGANISMS CONTAINED IN A MIXED SAMPLE WITH FACULTATIVE MICROORGANISMS
COMPOSITION DE MILIEU, PROCEDE ET DISPOSITIF PERMETTANT D'AUGMENTER DE MANIERE SELECTIVE L'ISOLEMENT DE MICRO-ORGANISMES %ANAEROBIES% CONTENUS DANS UN ECHANTILLON MELANGE PRESENTANT DES MICRO-ORGANISMES FACULTATIFS
Patent Applicant/Assignee:
  OXYRASE INC, P.O. Box 1345, Mansfield, OH 44901, US, US (Residence), US (Nationality)
Inventor(s):
  COPELAND James C, 298 N. Countryside Drive, Ashland, OH 44805, US,
  MYERS Kathy J, 269 Bowland Street, Mansfield, OH 44907, US,
Legal Representative:

(Glutamate-Ammonia Ligase)
Record Date Created: 19850816
Record Date Completed: 19850816

7/9/6 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

05338006 EMBASE No: 1993106091
Enzymes of ***anaerobic*** metabolism of phenolic compounds. 4-Hydroxybenzoyl-CoA reductase (dehydroxylating) from a denitrifying Pseudomonas species
Brackmann R.; Fuchs G.
Angewandte Mikrobiologie, Universitat Ulm, Postfach 4066,D-7900 Ulm Germany
European Journal of Biochemistry (EUR. J. BIOCHEM.) (Germany) 1993, 213/1 (563-571)
CODEN: EJBCA ISSN: 0014-2956
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

The reductive removal of aromatic hydroxyl functions plays an important role in the ***anaerobic*** metabolism of many phenolic compounds. We describe a new enzyme from a denitrifying Pseudomonas sp., 4-hydroxybenzoyl-CoA reductase (dehydroxylating), which reductively dehydroxylates 4-hydroxybenzoyl-CoA to benzoyl-CoA. The enzyme plays a role in the **anaerobic** degradation of phenol, 4-hydroxybenzoate, p-cresol, 4-hydroxyphenylacetate, and other aromatic compounds of which 4-hydroxybenzoyl-CoA is an intermediate. The enzyme is therefore induced only under anoxic conditions with these aromatic substrates, but not with benzoate or under aerobic conditions. A similar enzyme which reductively dehydroxylates 3-hydroxybenzoyl-CoA is induced during **anaerobic** growth with 3-hydroxybenzoate. The soluble enzyme 4-hydroxybenzoyl-CoA reductase was purified. It has a molecular mass of 260 kDa and consists of three subunits of 75, 35, and 17 kDa. The subunit composition is likely to be ainf 2binf 2cinf 2. The enzyme contains 12 mol iron/mol and 12 mol acid-labile sulfur/mol and exhibits a typical ultraviolet electron donor such as reduced viologen dyes; no other co-catalysts are required, the product is benzoyl-CoA and oxidized dye. The reductase is rapidly inactivated by oxygen. The inactivation by low concentrations of cyanide or **azide** in a pseudo-first-order time course suggests that it may contain a transition metal in an oxidation state which reacts with these ligands. 4-Hydroxybenzoyl-CoA reductase represents a type of enzyme which is common in ***anaerobic*** aromatic metabolism of phenolic compounds. A similar enzyme is demonstrated in Rhodopseupdomona palustris ***anaerobically*** grown with 4-hydroxybenzoate. The biological significance of reductive dehydroxylation of aromatics and a possible reaction mechanism similar to the Birch reduction are discussed.

DRUG DESCRIPTORS:
*aromatic compound; ***oxygenase**--endogenous compound--ec
phenol; unclassified drug
MEDICAL DESCRIPTORS:
*denitrification; *pseudomonas
anaerobic metabolism; article; nonhuman; priority journal
DRUG TERMS (UNCONTROLLED): 4 hydroxybenzoylcoenzyme a reductase--endogenous compound--ec
CAS REGISTRY NO.: 9037-29-0, 9046-59-7 (***oxygenase***); 108-95-2, 3229-70-7 (phenol
SECTION HEADINGS:
004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
?

7/9/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10630512 PMID: 8019429
Respiratory inhibitors activate an Fnr-like regulatory protein in Paracoccus denitrificans: implications for the regulation of the denitrification pathway.
Kucera I; Matchova I; Spiro S
Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic.
Biochemistry and molecular biology international (AUSTRALIA) Feb 1994, 32 (2) p245-50, ISSN 1039-9712 Journal Code: 9306673
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Unlike the uncoupler carbonyl cyanide 3-chlorophenyl-hydrazone, the respiratory inhibitors CN-, N3-, NO2- and rotenone enhanced the formation of nitrate and nitrite reductases in highly aerated cultures of the Paracoccus denitrificans ex-conjugant PD1222 (pRW2A/FF). A maximal effect was observed at concentrations partly blocking electron transport to O2. The level of beta-galactosidase reporting the activity of an Fnr-like regulatory protein showed a similar concentration dependency. It is concluded that oxygen is sensed by Fnr in an indirect way, possibly via the redox state of a cellular component.
Tags: Research Support, Non-U.S. Gov't
Descriptors: *Bacterial Proteins--metabolism--ME; *Escherichia coli Proteins; *Iron-Sulfur Proteins; *Oxygen Consumption--drug effects--DE; *Paracoccus denitrificans--chemistry--CH; *Paracoccus denitrificans--drug effects--DE; Aerobiosis; **Anaerobiosis**; **Azides**--pharmacology--PD ; Cyanides--pharmacology--PD; Electron Transport--drug effects--DE; Nitrate Reductases--biosynthesis--BI; Nitrate Reductases--metabolism--ME; Nitrite Reductases--biosynthesis--BI; Nitrite Reductases--metabolism--ME; Nitrogen --metabolism--ME; Nitrogen Dioxide--pharmacology--PD; Oxidation-Reduction; **Oxygenases**--antagonists and inhibitors--AI; **Oxygenases** --metabolism--ME; Paracoccus denitrificans--physiology--PH; Rotenone --pharmacology--PD; beta-Galactosidase--biosynthesis--BI; beta-Galactosida se--metabolism--ME
CAS Registry No.: 0 (Azides); 0 (Bacterial Proteins); 0 (Cyanides); 0 (Escherichia coli Proteins); 0 (FNR protein, E coli); 0 (Iron-Sulfur Proteins); 10102-44-0 (Nitrogen Dioxide); 7727-37-9 (Nitrogen); 83-79-4 (Rotenone)
Enzyme No.: EC 1. (Nitrite Reductases); EC 1.- (Nitrate Reductases); EC 1.13. (***Oxygenases***); EC 3.2.1.23 (beta-Galactosidase)
Record Date Created: 19940804
Record Date Completed: 19940804

7/9/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10341804 PMID: 8223558
Abduction of iron(III) from the soluble methane monooxygenase hydroxylase and reconstitution of the binuclear site with iron and manganese.
Atta M; Fontecave M; Wilkins P C; Dalton H
Laboratoire d'Etudes Dynamiques et Structurales de la Selectivite, URA Centre National de la Recherche Scientifique 0332, Universite J. Fourier, Grenoble, France.
European journal of biochemistry / FEBS (GERMANY) Oct 1 1993, 217 (1) p217-23, ISSN 0014-2956 Journal Code: 0107600

(6-aminoriboflavin); 83-88-5 (Riboflavin)
Enzyme No.: EC 1.- (Mixed Function ***Oxygenases***); EC 1.13.12.4 (lactate 2-monooxygenase); EC 1.4.3.3 (D-Amino-Acid Oxidase); EC 1.6.99.1 (NADPH Dehydrogenase)
Record Date Created: 19870320
Record Date Completed: 19870320

7/9/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07293340 PMID: 2861789
Inactivation of glutamine synthetase by a purified rabbit liver microsomal cytochrome P-450 system.
Nakamura K; Oliver C; Stadtman E R
Archives of biochemistry and biophysics (UNITED STATES) Jul 1985, 240 (1) p319-29, ISSN 0003-9861 Journal Code: 0372430
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Several mixed-function oxidation systems catalyze inactivation of Escherichia coli glutamine synthetase and other key metabolic enzymes. In the presence of NADPH and molecular oxygen, highly purified preparations of cytochrome P-450 reductase and cytochrome P-450 (isozyme 2) from rabbit liver microsomes catalyze enzyme inactivation. The inactivation reaction is stimulated by Fe(III) or Cu(II) and is inhibited by catalase, Mn(II), Zn(II), histidine, and the metal chelators o-phenanthroline and EDTA. The inactivation of glutamine synthetase is highly specific and involves the oxidative modification of a histidine in each glutamine synthetase subunit and the generation of a carbonyl derivative of the protein which forms a stable hydrazone when treated with 2,4-dinitrophenylhydrazine. We have proposed that the mixed-function oxidation system (the cytochrome P-450 system) produces Fe(II) and H2O2 which react at the metal binding site on the glutamine synthetase to generate an activated oxygen species which oxidizes a nearby susceptible histidine. This thesis is supported by the fact that (a) Mn(II) and Zn(II) inhibit inactivation and also interfere with the reduction of Fe(III) to Fe(II) by the P-450 system; (b) Fe(II) and H2O2 (**anaerobically**), in the absence of a P-450 system, catalyze glutamine synthetase inactivation; (c) inactivation is inhibited by catalase; and (d) hexobarbital, which stimulates the rate of H2O2 production by the P-450 system, stimulates the rate of glutamine synthetase inactivation. Moreover, inactivation of glutamine synthetase by the P-450 system does not require complex formation because inactivation occurs when the P-450 components and the glutamine synthetase are separated by a semipermeable membrane. Also, if endogenous catalase is inhibited by **azide**, rabbit liver microsomes catalyze the inactivation of glutamine synthetase.
Tags: In Vitro
Descriptors: *Cytochrome P-450 Enzyme System--metabolism--ME; *Glutamate-Ammonia Ligase--metabolism--ME; Animals; Binding Sites; Catalysis; Cell Membrane Permeability; Enzyme Induction--drug effects--DE; Escherichia coli--enzymology--EN; Hydrogen Peroxide--metabolism--ME; Iron --metabolism--ME; Microsomes, Liver--enzymology--EN; Mixed Function **Oxygenases**--metabolism--ME; Oxidation-Reduction; Phenobarbital --pharmacology--PD; Rabbits; Substrate Specificity; Vitamin K--pharmacology --PD
CAS Registry No.: 12001-79-5 (Vitamin K); 50-06-6 (Phenobarbital); 7439-89-6 (Iron); 7722-84-1 (Hydrogen Peroxide); 9035-51-2 (Cytochrome P-450 Enzyme System)
Enzyme No.: EC 1.- (Mixed Function ***Oxygenases***); EC 6.3.1.2

Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

The apo-form of the soluble methane monooxygenase hydroxylase from Methylococcus capsulatus (Bath) was prepared via chelation of iron(III) with 3,4-dihydroxybenzaldehyde. The apohydroxylase was reconstituted by the ***anaerobic*** addition of Fe(II) followed by air oxidation. The enzyme thus prepared regained 85-90% of its original catalytic activity. The incorporation of two manganese(II) ions/mol of apohydroxylase was monitored by EPR spectroscopy. The Mn(II) ions occupy the native diiron active site and remain in the +2 oxidation state. The EPR data suggest strong coupling between the two Mn(II) ions and retention of the mu-hydroxo (alkoxo) bridge. The results of this study indicate that the M. capsulatus (Bath) hydroxylase contains a single diiron site.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Ferric Compounds--chemistry--CH; *Ferrous Compounds --chemistry--CH; *Manganese--chemistry--CH; ***Oxygenases**--chemistry --CH; Apoenzymes--chemistry--CH; Apoenzymes--metabolism--ME; **Azides** --pharmacology--PD; Benzaldehydes; Binding Sites; Catalysis; Catechols; Chelating Agents; Electron Spin Resonance Spectroscopy; Methylococcaceae --enzymology--EN; Mixed Function **Oxygenases**--chemistry--CH; Mixed Function **Oxygenases**--metabolism--ME; Oxidation-Reduction; **Oxygenas es**--metabolism--ME; Structure-Activity Relationship

CAS Registry No.: 0 (Apoenzymes); 0 (Azides); 0 (Benzaldehydes); 0 (Catechols); 0 (Chelating Agents); 0 (Ferric Compounds); 0 (Ferrous Compounds); 139-85-5 (protocatechualdehyde); 7439-96-5 (Manganese)

Enzyme No.: EC 1.- (Mixed Function ***Oxygenases***); EC 1.13. (***Oxygenases***); EC 1.14.13.25 (methane monooxygenase)

Record Date Created: 19931203
Record Date Completed: 19931203

7/9/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08191201 PMID: 3275880

Inhibitors of N-nitroso compounds-induced mutagenicity.

Gichner T; Veleminsky J

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha 5.

Mutation research (NETHERLANDS) Jan 1988, 195 (1) p21-43, ISSN 0027-5107 Journal Code: 0400763

Publishing Model Print
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

N-Nitroso compounds are environmental mutagens that are present in the air, water, soil etc. or can be formed by nitrosation of various nitrosatable compounds. The present paper gives a survey of inhibitors of N-nitroso compounds-induced mutagenicity. Inhibitors covered include: thiols, metals, vitamins, phenolic acids, complex mixtures of plant, animal and human origin, organic solvents, inhibitors of mixed-function oxidases etc. Data on inhibitors that prevent the formation of N-nitroso compounds are not covered in this review. (220 Refs.)

Descriptors: *Nitroso Compounds--antagonists and inhibitors--AI; 4-Aminobenzoic Acid--pharmacology--PD; **Anaerobiosis**; Animals; Antioxidants--pharmacology--PD; **Azides**--pharmacology--PD; Body Fluids ; Food Analysis; Humans; Metals--pharmacology--PD; Mixed Function

Oxygenases--antagonists and inhibitors--AI; Mutagenicity Tests; Nitroso Compounds--pharmacology--PD; Phenols--pharmacology--PD; Plant Extracts--pharmacology--PD; Sodium **Azide**; Solvents--pharmacology--PD; Sulfhydryl Compounds--pharmacology--PD; Tissue Extracts--pharmacology--PD; Vitamins--pharmacology--PD

CAS Registry No.: 0 (Antioxidants); 0 (Azides); 0 (Metals); 0 (Nitroso Compounds); 0 (Phenols); 0 (Plant Extracts); 0 (Solvents); 0 (Sulfhydryl Compounds); 0 (Tissue Extracts); 0 (Vitamins); 150-13-0 (4-Aminobenzoic Acid); 26628-22-8 (Sodium Azide)

Enzyme No.: EC 1.- (Mixed Function ***Oxygenases***)

Record Date Created: 19880217

Record Date Completed: 19880217

7/9/4 (Item 4 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

07768966 PMID: 2879563

6-Azido- and 6-aminoflavins as active-site probes of flavin enzymes.

Massey V; Ghisla S; Yagi K

Biochemistry (UNITED STATES) Dec 2 1986, 25 (24) p8095-102, ISSN 0006-2960 Journal Code: 0370623

Contract/Grant No.: GM 11106; GM; NIGMS

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

6-Azidoflavins have been bound to the apoproteins of five representative flavoproteins and their properties, before and after light irradiation, compared with those of the same proteins containing the appropriate 6-aminoflavin. In the dark the 6-azidoflavoproteins are quite stable, except for L-lactate oxidase, where spontaneous conversion to the 6-amino-FMN enzyme occurs slowly at pH 7. 6-Azido-FMN Old Yellow Enzyme is converted to the 6-amino-FMN enzyme by aerobic turnover with NADPH, and 6-azido-FAD D-amino acid oxidase is converted to the 6-amino-FAD enzyme by treatment with D-alanine. Light irradiation of 6-azidoriboflavin bound to riboflavin-binding protein does not result in any covalent fixation of the flavin to the protein. Light irradiation of 6-azido-FMN flavodoxin gives only a small amount of covalent linkage. In contrast, 6-azido-FMN Old Yellow Enzyme undergoes a very facile light-induced change, in which approximately 50% of the flavin is attached in a stable covalent linkage to the protein. The resulting flavoprotein form has lost the ability to bind phenols, a distinctive characteristic of the native enzyme; it does, however, bind NADPH, but the latter cannot reduce the covalently bound flavin. 6-Azido-FAD D-amino acid oxidase also undergoes a facile light modification, in which almost quantitative fixation of the flavin to the protein takes place. The resulting flavoprotein cannot bind benzoate, an active-site ligand for the native enzyme, nor is it reduced ***anaerobically*** by D-alanine. The covalent linkage is fairly labile and is destroyed on denaturation of the protein.(ABSTRACT TRUNCATED AT 250 WORDS)

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: ***Azides**--pharmacology--PD; *Flavins--pharmacology--PD; *Flavoproteins--metabolism--ME; *Riboflavin--analogs and derivatives--AA; Binding Sites; D-Amino-Acid Oxidase--metabolism--ME; Flavodoxin--metabolism --ME; Kinetics; Mixed Function **Oxygenases**--metabolism--ME; NADPH Dehydrogenase--metabolism--ME; Riboflavin--pharmacology--PD; Spectrophotome try

CAS Registry No.: 0 (Azides); 0 (Flavins); 0 (Flavodoxin); 0 (Flavoproteins); 101760-83-2 (6-azidoriboflavin); 73652-55-8

J Clin Pathol. 1980 Jan;33(1):61-5. Related Articles, Links

Multiple selective media for the isolation of anaerobic bacteria from clinical specimens.

Wren MW.

Using fresh clinical material, a comparison of a number of anaerobic selective media was made. For Gram-negative anaerobes nalidixic acid tween agar (NAT), neomycin agar (NA), and neomycin-vancomycin agar (NVA) all performed equally well. Kanamycin-containing media were more inhibitory to all Gram-negative anaerobes other than Bacteroides fragilis and B. melaninogenicus. When the recovery of Gram-positive anaerobes was examined NAT performed better than any of the other selective media used. No single selective medium could recover all anaerobes. Better isolation was achieved using a combination of two selective media (the best combinations being NAT and NVA or NAT and NA). Only a combination of three selective media gave the maximum recovery of anaerobes in this study (NAT, NVA, and NA or KA).

PMID: 6987278 [PubMed - indexed for MEDLINE]

13/9/1
DIALOG(R)File 155:MEDLINE(R)

10206026 PMID: 8390371
Mitochondrial heterogeneity in the parasitic nematode, Ascaris suum.
Komuniecki P R; Johnson J; Kamhawi M; Komuniecki R
Department of Biology, University of Toledo, Ohio 43606-3390.
Experimental parasitology (UNITED STATES) Jun 1993, 76 (4) p424-37, ISSN 0014-4894 Journal Code: 0370713
Contract/Grant No.: AI 18427; AI; NIAID
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The **anaerobic** metabolism of Ascaris suum body wall muscle **mitochondria** has been well characterized, but little is known about the metabolism of other adult tissues. The present study was designed to further characterize the metabolism of **mitochondria isolated** from A. suum male reproductive tissues, which contain predominately sperm, and to compare it with that of muscle. Cytochrome oxidase activity could not be detected in muscle, testis, or sperm **mitochondria** either by diaminobenzidine staining or enzymatic assays. However, the activities of several tricarboxylic acid cycle enzymes, including citrate synthase and isocitrate dehydrogenase, were about 100-fold higher in testis/seminal vesicle ***mitochondria*** than muscle ***mitochondria*** . In contrast, malic enzyme activity in testis/seminal vesicle **mitochondria** was about 12-fold lower than that in muscle ***mitochondria*** . The incorporation of 32Pi into organic phosphate by either muscle or testis/seminal vesicle **mitochondria** appeared to be dependent on malate and pyruvate, and incorporation was inhibited by rotenone but not ***cyanide*** . Thus, the metabolism of testis/seminal vesicle **mitochondrial** preparations appears to be similar to that of ascarid muscle, despite the elevated levels of tricarboxylic acid cycle enzyme activities present in testis/seminal vesicle ***mitochondria*** . The function of these elevated enzymes is unclear, but the possibility that they are used later in the aerobic metabolism of the fertilized egg has not been excluded.
Tags: Comparative Study; Male; Research Support, U.S. Gov't, P.H.S.
Descriptors: *Ascaris suum--ultrastructure--UL; ***Mitochondria** --metabolism--ME; Animals; Ascaris suum--chemistry--CH; Ascaris suum --metabolism--ME; Cell **Fractionation**; Citric Acid Cycle; Electron Transport Complex IV--metabolism--ME; Helminth Proteins--analysis--AN; Malate Dehydrogenase--metabolism--ME; Microscopy, Electron; **Mitochondria** --chemistry--CH; **Mitochondria**--ultrastructure--UL; **Mitochondri** **a**, Muscle--chemistry--CH; **Mitochondria**, Muscle--metabolism--ME; **Mitochondria**, Muscle--ultrastructure--UL; Phosphates--metabolism--ME; Phosphorylation; Spermatozoa--metabolism--ME; Spermatozoa--ultrastructure --UL; Testis--metabolism--ME; Testis--ultrastructure--UL
CAS Registry No.: 0 (Helminth Proteins); 0 (Phosphates)
Enzyme No.: EC 1.1.1.37 (Malate Dehydrogenase); EC 1.9.3.1 (Electron Transport Complex IV)
Record Date Created: 19930719
Record Date Completed: 19930719

13/9/2
DIALOG(R)File 155:MEDLINE(R)

07641610 PMID: 2943272

detergent-containing cytochrome o is composed of one polypeptide chain with a molecular weight of 28 000-29 000, associated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The enzyme exists as a dimer by gel filtration analysis. The amino analysis which reveals the majority of residues are of hydrophobic nature. The cytochrome o oxidase contains protoheme as its prosthetic group and about 20-40% of phospholipids. The phospholipids are identified as phosphatidylethanolamine and phosphatidylglycerol by radioautographic analysis using 2-dimensional thin-layer chromatography. No copper or nonheme iron can be detected in the purified oxidase preparation by atomic absorption and chemical analyses. Oxidation-reduction titration shows this **membrane**-bound cytochrome o to be a low-potential component, and Em was determined to be -18 mV in the purified form and -30 mV in the ***membrane*** -bound form. Both forms bind CO with a reduced absorption peak at 559 and 557-558 nm in the native and solubilized forms, respectively. A high-spin (g = 6.0) form is assigned to the oxidized cytochrome o by electron paramagnetic resonance analysis, and KCN abolishes this high-spin signal. CO titration of purified cytochrome o in the **anaerobic** conditions shows the enzyme binds one CO per four protohemes and a dissociation constant is estimated to be 3.2 microM for CO. ***Cyanide*** reacts with purified cytochrome o in both oxidized and CO-bound forms, identified by specific spectral compounds absorbed at the Soret region. Cytochrome c, often co-purified with cytochrome c from the **membrane** , cannot serve as a reductant for cytochrome o in vitro, due to the apparent potential difference of about 300 mV. Upon separation, both cytochrome o and cytochrome c4 show a great tendency of aggregation. Furthermore, the oxidase activity (measured by tetramethyl-p-phenylenediami ne oxidation rate) decreases as the cytochrome c concentration is decreased by ammonium sulfate ***fractionation*** . All these suggest the structural and functional complex nature of cytochrome c4 and cytochrome o in the ***membrane*** of A. vinelandii.

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Azotobacter--enzymology--EN; *Cytochrome b Group; *Cytochromes; *Escherichia coli Proteins; Carbon Monoxide; Cytochromes--**isolation** and purification--IP; Detergents; **Membranes** --enzymology--EN; Molecular Weight; Osmolar Concentration; Phospholipids --analysis--AN; Solubility; Spectrum Analysis

CAS Registry No.: 0 (Cytochrome b Group); 0 (Cytochromes); 0 (Detergents); 0 (Escherichia coli Proteins); 0 (Phospholipids); 630-08-0 (Carbon Monoxide); 9035-48-7 (cytochrome bo, E coli)

Record Date Created: 19860409

Record Date Completed: 19860409

13/9/4

DIALOG(R)File 155:MEDLINE(R)

07357199 PMID: 2995315

Participation of cytochromes in some oxidation-reduction systems in Campylobacter fetus.

Lascelles J; Calder K M

Journal of bacteriology (UNITED STATES) Oct 1985, 164 (1) p401-9, ISSN 0021-9193 Journal Code: 2985120R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Campylobacter species are rich in c-type cytochromes, including forms which bind carbon monoxide. The role of the various forms of cytochromes in Campylobacter fetus has been examined in cell-free preparations by using physiological electron donor and acceptor systems. Under ***anaerobic***

conditions, NADPH reduced essentially all of the cytochrome c in crude cell extracts, whereas the reduction level with succinate was 50 to 60%. The carbon monoxide spectrum with NADPH was predominated by the cytochrome c complex; evidence of a cytochrome o type was seen in the succinate-reduced extracts and in ***membrane*** ***fractions*** . Succinate-reduced cytochrome c was oxidized by oxygen via a **cyanide**-sensitive, ***membrane*** -associated system. NADPH-reduced cytochrome c was oxidized by a ***cyanide*** -insensitive system. Partially purified carbon monoxide-binding cytochrome c, **isolated** from the cytoplasm, could serve as electron acceptor for NADPH-cytochrome c oxidoreductase; the reduced cytochrome was oxidized by oxygen by a **cyanide**-insensitive system present in the cytoplasmic ***fraction*** . Horse heart cytochrome c was also reducible by NADPH and by succinate; the reduced cytochrome was oxidized by a **cyanide**-sensitive system in the **membrane** ***fraction*** . NADPH and NADH oxidase activities were observed aerobically and under ***anaerobic*** conditions with fumarate. NADPH was more active than NADH. NADP was also more effective than NAD as an electron acceptor for the coenzyme A-dependent pyruvate and alpha-ketoglutarate dehydrogenase activities found in crude extracts. These dehydrogenases used methyl viologen and metronidazole as electron acceptors; they could be loci for oxygen inhibition of growth. It is proposed that energy provision via the high-potential cytochrome c oxidase system in the cytoplasmic **membrane** is limited by oxygen-sensitive primary dehydrogenases and that the carbon monoxide-binding cytochrome c may have a role as an ***oxygen*** ***scavenger*** .

Tags: Research Support, U.S. Gov't, Non-P.H.S.

Descriptors: *Campylobacter fetus--metabolism--ME; *Cytochromes --physiology--PH; Cell **Membrane**--enzymology--EN; Citric Acid Cycle; Cytochrome c Group--metabolism--ME; Cytoplasm--enzymology--EN; Ketoglutarate Dehydrogenase Complex--analysis--AN; Multienzyme Complexes --analysis--AN; NADH, NADPH Oxidoreductases--analysis--AN; NADP--metabolism --ME; NADPH Oxidase; Oxidation-Reduction; Oxygen--metabolism--ME; Pyruvate Dehydrogenase Complex--analysis--AN; Succinates--metabolism--ME; Succinic Acid

CAS Registry No.: 0 (Cytochrome c Group); 0 (Cytochromes); 0 (Multienzyme Complexes); 0 (Pyruvate Dehydrogenase Complex); 0 (Succinates); 110-15-6 (Succinic Acid); 53-59-8 (NADP); 7782-44-7 (Oxygen)

Enzyme No.: EC 1.2.4.2 (Ketoglutarate Dehydrogenase Complex); EC 1.6. (NADH, NADPH Oxidoreductases); EC 1.6.- (NADH2 oxidase); EC 1.6.- (NADPH Oxidase)

Record Date Created: 19851114

Record Date Completed: 19851114

13/9/5

DIALOG(R)File 155:MEDLINE(R)

07231792 PMID: 3990708

Respiration and energy conservation in the filarial worm Litomosoides carinii.

Ramp T; Bachmann R; Kohler P

Molecular and biochemical parasitology (NETHERLANDS) Apr 1985, 15 (1) p11-20, ISSN 0166-6851 Journal Code: 8006324

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The average rate of endogenous respiration of intact Litomosoides carinii was 2.24 muatom O min-1 g-1 worm wet wt. No significant difference was observed in respiration capacities between male and female worms. Rates of

oxygen uptake decreased progressively during disruption and **fractionation** of the parasite tissue and very few respiration capabilities remained in the ***mitochondrial*** ***fraction*** . Added substrates increased the respiratory rates of the intact filariid and cell-free extracts by a factor of 1.4 to 2.3, depending on the tissue system and substrate species used. Rotenone and ***cyanide*** strongly inhibited respiration in all incubations, whereas antimycin A, in most cases, suppressed oxygen consumption only partially. ATP conservation in cell-free extracts of L. carinii, as determined by the incorporation of 32Pi into the organic phosphate **fraction**, was twice as high in the presence of air as under an atmosphere of nitrogen. ***Anaerobically*** , rates of phosphorylation in these extracts were similar to the amounts of lactate. Phosphorylation in ***mitochondria*** ***isolated*** from the filarial worm was supported by malate, succinate, pyruvate and TMPD/ascorbate, whereas L-glutamate and beta-hydroxybutyrate exhibited only little or no effect, respectively. P/O ratios for pyruvate-supported oxidative phosphorylation were found to approach a value of 3. Electron transport inhibitors, oligomycin and 2,4-dinitrophenol strongly inhibited substrate-dependent ***mitochondrial*** phosphorylation. The data of the present investigation, together with other recent findings made by the same authors, have provided evidence that in L. carinii ***mitochondria*** a mammalian-type of respiratory system capable of carrying out oxidative phosphorylation is functional. It seems likely that this respiration-dependent chemical energy, proceeding in addition to that generated through fermentation processes, may be vital for muscular contraction and survival of this filarial parasite.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Filarioidea--metabolism--ME; Adenosine Triphosphate --metabolism--ME; Aerobiosis; Animals; Energy Metabolism; **Mitochondria**--metabolism--ME; Oxidative Phosphorylation; Oxygen Consumption

CAS Registry No.: 56-65-5 (Adenosine Triphosphate)

Record Date Created: 19850619

Record Date Completed: 19850619

13/9/6

DIALOG(R)File 155:MEDLINE(R)

06338838 PMID: 6806238

Properties of dissimilatory nitrate reductase purified from the denitrifier Pseudomonas aeruginosa.

Carlson C A; Ferguson L P; Ingraham J L

Journal of bacteriology (UNITED STATES) Jul 1982, 151 (1) p162-71, ISSN 0021-9193 Journal Code: 2985120R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Dissimilatory nitrate reductase was purified to homogeneity from **anaerobic** cultures of the denitrifying bacterium Pseudomonas aeruginosa. The following procedures were used in the rapid ***isolation*** of this unstable enzyme: induction by nitrate in semianaerobic cell suspension, heat-stimulated activation and solubilization from the **membrane fraction**, and purification by hydrophobic interaction chromatography. The molecular weight of the purified enzyme was estimated by nondenaturing polyacrylamide gel electrophoresis, sucrose density gradient sedimentation, and gel filtration chromatography. Subunit molecular weights were estimated by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels. The active enzyme monomer, with a molecular weight of 176,000 to 260,000 (depending upon the method of determination),

was composed of subunits with molecular weights of approximately 64,000 and 118,000. The monomer aggregated to form an inactive tetramer of about 800,000 molecular weight. Purified enzyme exhibited a broad pH optimum, between 6.5 and 7.5. Kinetic studies showed that the apparent Km was 0.30 mM for nitrate, and 2.2 to 2.9 microM for dithionite-reduced benzyl viologen. ***Azide*** was an effective inhibitor: the concentration required for half-maximal inhibition was 21 to 24 microM. ***Azide*** inhibition was competitive with nitrate (Ki = 2.0 microM) but uncompetitive with reduced benzyl viologen (Ki = 25 microM). Based upon spectral evidence, the purified molybdo-enzyme had no associated cytochromes but did contain nonhaem iron that responded to dithionite reduction and nitrate oxidation. The enzyme that was purified after being heat solubilized from **membranes** had properties essentially identical to those of the enzyme that was purified after deoxycholate solubilization.

Tags: Research Support, U.S. Gov't, Non-P.H.S.

Descriptors: *Nitrate Reductases--metabolism--ME; *Pseudomonas aeruginosa --enzymology--EN; **Azides**--pharmacology--PD; Cell **Membrane** --enzymology--EN; Kinetics; Macromolecular Substances; Molecular Weight; Nitrate Reductases--**isolation** and purification--IP; Temperature

CAS Registry No.: 0 (Azides); 0 (Macromolecular Substances)

Enzyme No.: EC 1.- (Nitrate Reductases)

Record Date Created: 19820826

Record Date Completed: 19820826

13/9/7

DIALOG(R)File 155:MEDLINE(R)

05651178 PMID: 41594

Studies on the mobilization of iron from ferritin by **isolated** rat liver ***mitochondria*** .

Ulvik R; Romslo I

Biochimica et biophysica acta (NETHERLANDS) Dec 3 1979, 588 (2) p256-71, ISSN 0006-3002 Journal Code: 0217513

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Rat liver **mitochondria** and rat liver mitoplasts mobilize iron from ferritin by a mechanism which depends on a respiratory substrate (preferentially succinate), a small molecular weight electron mediator (FMN, phenazine methosulphate or methylene blue) and (near) **anaerobic** conditions. The release process under optimized conditions (approx. 50 mumol/l FMN, 1 mmol/l succinate, 0.35 mmol/l Fe(III) (as ferritin iron), 37 degrees C and pH 7.40) amounts to 0.9--1.2 nmol iron/mg protein per min. The results suggest that ferritin might function as an intermediate in the cytosolic transport of iron to the ***mitochondria*** .

Descriptors: *Ferritin--metabolism--ME; *Iron--metabolism--ME; *Mitochondria, Liver--metabolism--ME; Animals; Antimycin A --pharmacology--PD; Carboxylic Acids--pharmacology--PD; **Cyanides** --pharmacology--PD; Dyes; Hydrogen-Ion Concentration; NAD--pharmacology--PD; Oxidation-Reduction; Oxygen--pharmacology--PD; Phenanthrolines --pharmacology--PD; Rats; Rotenone--pharmacology--PD; Subcellular **Fractions**--metabolism--ME; Temperature

CAS Registry No.: 0 (Carboxylic Acids); 0 (Cyanides); 0 (Dyes); 0 (Phenanthrolines); 53-84-9 (NAD); 642-15-9 (Antimycin A); 7439-89-6 (Iron); 7782-44-7 (Oxygen); 83-79-4 (Rotenone); 9007-73-2 (Ferritin)

Record Date Created: 19800226

Record Date Completed: 19800226

13/9/8
DIALOG(R)File 155:MEDLINE(R)

05136467 PMID: 413543
The respiratory chain of a newly ***isolated*** Methylomonas Pl1.
Drabikowska A K
Biochemical journal (ENGLAND) Nov 15 1977, 168 (2) p171-8, ISSN 0264-6021 Journal Code: 2984726R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
1. Whole cells of Methylomonas Pl1 contained ubiquinone, identified as ubiquinone-8. No naphthaquinone was detected. Ubiquinone was located predominantly in the particulate **fraction**, which also contained most of the NADH oxidase activity. 2. Aerobic incubation of cells with formaldehyde or methanol resulted in about 20% reduction of ubiquinone, irrespective of the presence or absence of dinitrophenol. On inhibition of the respiration by **cyanide** , ubiquinone became partly reduced by endogenous substrates (15--25%), and a further reduction occurred only in the presence of formaldehyde (up to 60%). When endogenous substrates were completely exhausted, then 44 and 23% of ubiquinone was reduced by formaldehyde or methanol respectively. 3. The difference spectra at room and liquid-N2 temperatures revealed the presence of cytochrome b and two cytochromes c (c-552.5 and c-549) all tightly bound to the ***membrane*** . Cytochrome c-552.5 was also found in the soluble ***fraction*** . 4. Redox changes of cytochromes b and c, with methanol or formaldehyde as substrates, respond to the aerobic and **anaerobic** states of the cell and to KCN inhibition in a manner characteristic of the electron carriers of the respiratory chain. 5. The merging point for electron transport from NADH dehydrogenase and formaldehyde dehydrogenase is suggested to be at the level of ubiquinone.
Descriptors: *Methylococcaceae--metabolism--ME; Chromatography, Thin Layer; Cytochromes--metabolism--ME; Formaldehyde--metabolism--ME; Methanol --metabolism--ME; Oxidation-Reduction; Oxygen Consumption; Quinones --metabolism--ME; Spectrum Analysis; Ubiquinone--metabolism--ME
CAS Registry No.: 0 (Cytochromes); 0 (Quinones); 1339-63-5 (Ubiquinone); 50-00-0 (Formaldehyde); 67-56-1 (Methanol)
Record Date Created: 19780223
Record Date Completed: 19780223

13/9/9
DIALOG(R)File 155:MEDLINE(R)

04915710 PMID: 838730
Effect of ***membrane*** environment on succinate dehydrogenase activity.
Ackrell B A; Kearney E B; Singer T P
Journal of biological chemistry (UNITED STATES) Mar 10 1977, 252 (5) p1582-8, ISSN 0021-9258 Journal Code: 2985121R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The turnover number of succinate dehydrogenase from mammalian heart determined by the spectrophotometric phenazine methosulfate assay, after complete activation, is approximately 21,000 mol of succinate oxidized/min/mol of histidyl flavin at 38 degrees in relatively intact

inner ***membrane*** preparations and ***mitochondria*** . Reconstitutively active soluble preparations, extracted **anaerobically** in the presence of succinate from inner **membrane** preparations show turnover numbers of 11,500 to 14,500 and a significantly lower apparent Km for phenazine methosulfate than the parent particles. The decline of both the turnover number and of the Km occurs during the brief period when the enzyme is detached from the ***membrane*** . The observed values represent the activities in the soluble extract of both the reconstitutively active and reconstitutively inactive enzyme. The latter may be from 10 to 40% even in the most carefully prepared enzyme; it has a lower turnover number in the phenazine methosulfate assay than the average for the solution and is devoid of catalytic activity in the "low Km" ferricyanide assay (Vinogradov, A. D., Ackrell, B.A.C., and Singer, T.P. (1975) Biochem. Biophys. Res. Commun. 67, 803-809). The reconstitutively active form of the soluble enzyme has a turnover number of at least 15,000 and an equal activity in the low Km ferricyamide assay. When recombined with the **membrane** the total activity of the enzyme is increased by over 60% and it regains the original turnover number, Km for phenazine methosulfate, and sensitivity of the phenazine methosulfate reductase activity to thenoyltrifluoroacetone, carboxamides, and ***cyanide*** . It appears, therefore, that the **membrane** environment or some component of it exerts a positive modulating influence on the enzyme even in the fully activated state. In certain particulate sources (Keilin-Hartree preparations, Complex II) the enzyme shows lower turnover numbers (11,000 to 12,500) than in more intact inner ***membranes*** . This seems to be due to inactivation in the course of preparation and, in the case of Complex II, in part also to loss of the normal **membrane** environment or of a ***membrane*** component, possibly Q-10, during ***isolation*** .

Tags: Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.

Descriptors: ***Mitochondria**, Muscle--enzymology--EN; *Myocardium --enzymology--EN; *Succinate Dehydrogenase--metabolism--ME; Animals; Binding Sites; Cattle; Cell **Fractionation**; Cell **Membrane** --enzymology--EN; Kinetics; **Membranes**--enzymology--EN; Methylphenazonium Methosulfate--pharmacology--PD; Protein Binding

CAS Registry No.: 299-11-6 (Methylphenazonium Methosulfate)

Enzyme No.: EC 1.3.99.1 (Succinate Dehydrogenase)

Record Date Created: 19770415

Record Date Completed: 19770415

13/9/10

DIALOG(R)File 155:MEDLINE(R)

04540945 PMID: 1099093

The purification and properties of formate dehydrogenase and nitrate reductase from Escherichia coli.

Enoch H G; Lester R L

Journal of biological chemistry (UNITED STATES) Sep 10 1975, 250 (17) p6693-705, ISSN 0021-9258 Journal Code: 2985121R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The **membrane**-bound formate dehydrogenase of Escherichia coli grown **anaerobically** in the presence of nitrate was solubilized with deoxycholate and purified to near homogeneity. The purification procedure included ammonium sulfate **fractionation** and chromatography on Bio-Gel A-1.5m and DEAE Bio-Gel A in the presence of the nonionic detergent, Triton X-100. This detergent caused a significant decrease in the molecular weight of the soluble formate dehydrogenase complex and allowed the enzyme then to

be resolved from other ***membrane*** components. ***Anaerobic*** conditions were required throughout due to the sensitivity of the enzyme to oxygen inactivation. Formate dehydrogenase was judged to be at least 93 to 99% pure by the following procedures: polyacrylamide gel electrophoresis in the presence of Triton X-100 and sodium dodecyl sulfate, gel filtration, and sedimentation velocity studies. The purified enzyme exists as a detergent-protein complex (0.20 +/- 0.03 g of Triton X-100/g of protein) which has an S20,w of 18.1 S and a Stokes radius of 76 A. This corresponds to a molecular weight of 590,000 +/- 59,000. The enzyme had an absorbance spectrum of a b-type cytochrome which could be completely reduced by formate. The heme content corresponds to an equivalent weight of 154,000 which suggests a tetrameric structure for the enzyme. Formate dehydrogenase was found to contain (in relative molar amounts): 1.0 heme, 0.95 molybdenum, 0.96 selenium, 14 non-heme iron, and 13 acid-labile sulfide. Neither FAD nor FMN could be detected. The enzyme contains three polypeptides, designated alpha, beta, and gamma, whose molecular weights were estimated by gel electrophoresis in the presence of sodium dodecyl sulfate to be 110,000, 32,000, and 20,000, respectively. After separation of the polypeptides by gel filtration in the presence of sodium dodecyl sulfate alpha, beta, and gamma were found in 1:1.2:0.55 molar ratios. A study of the enzyme obtained from cells grown with [75Se]selenite showed that only the alpha polypeptide contained significant amounts of selenium. The enzyme will catalyze the formate-dependent reduction of phenazine methosulfate, dichlorophenolindophenol, methylene blue, nitroblue tetrazolium, benzyl viologen, methyl viologen, ferricyanide, and coenzyme Q6. ***Cyanide*** , ***azide*** , p-hydroxymercuribenzoate, iodoacetamide, and oxygen inhibit the enzyme. The procedure which was designed for the purification of formate dehydrogenase also yields a highly purified preparation of nitrate reductase. This nitrate reductase has been shown to contain significant amounts of heme (Enoch, H. G., and Lester, R. L. (1974) Biochem. Biophys. Res Commun. 61,1234-1241). The enzyme contains three polypeptides with molecular weights of 155,000, 63,000, and 19,000. When measured in the presence of Trition X-100 the Stokes radius of nitrate reductase is 75 A and the S20,w is 16 S which corresponds to a molecular weight of 498,000.

Tags: Research Support, U.S. Gov't, P.H.S.

Descriptors: *Aldehyde Oxidoreductases--**isolation** and purification --IP; *Escherichia coli--enzymology--EN; *Nitrate Reductases--**isolation** and purification--IP; Aldehyde Oxidoreductases--metabolism --ME; Binding Sites; Cell **Membrane**--enzymology--EN; Electron Transport; Formates; Kinetics; Macromolecular Substances; Molecular Weight; Molybdenum--analysis--AN; Nitrate Reductases--metabolism--ME; Phospholipids --analysis--AN; Protein Conformation

CAS Registry No.: 0 (Formates); 0 (Macromolecular Substances); 0 (Phospholipids); 7439-98-7 (Molybdenum)

Enzyme No.: EC 1.- (Nitrate Reductases); EC 1.2. (Aldehyde Oxidoreductases)

Record Date Created: 19751204

Record Date Completed: 19751204

Regulation of Na+ transport in brown adipose tissue.
LaNoue K F; Koch C; Strzelecka D; Kobylski T P
Biochemical journal (ENGLAND) Apr 15 1986, 235 (2) p545-52, ISSN 0264-6021 Journal Code: 2984726R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
In order to test the hypothesis that Na+, K+-ATPase (Na+,K+-dependent ATPase) is involved in the noradrenaline-mediated stimulation of respiration in brown adipose tissue, the effects of noradrenaline on Na+,K+-ATPase in **isolated** brown-fat-cell **membrane** vesicles, and on 22Na+ and K+ (86Rb+) fluxes across the **membranes** of intact ***isolated*** cells, were measured. The ouabain-sensitive ***fraction*** of the K+-dependent ATPase activity in the **isolated membrane** -vesicle preparation was small and was not affected by the presence of noradrenaline in the incubation media. The uptake of 86Rb+ into intact hormone-sensitive cells was inhibited by 80% by ouabain, but it was insensitive to the presence of noradrenaline. 22Na+ uptake and efflux measured in the intact cells were 8 times more rapid than the 86Rb+ fluxes and were unaffected by ouabain. This indicated the presence of a separate, more active, transport system for Na+ than the Na+,K+-ATPase. This is likely to be a Na+/Na+ exchange activity under normal aerobic conditions. However, under **anaerobic** conditions, or conditions simulating anaerobiosis (2 mM-NaCN), the unidirectional uptake of Na+ increased dramatically, while efflux was unaltered.
Descriptors: *Brown Fat--metabolism--ME; *Sodium--metabolism--ME; Adenosinetriphosphatase--metabolism--ME; Animals; Biological Transport; Brown Fat--cytology--CY; Brown Fat--drug effects--DE; Calcium --pharmacology--PD; Cell **Membrane**--metabolism--ME; **Cyanides** --pharmacology--PD; Hamsters; Iodoacetates--pharmacology--PD; Iodoacetic Acid; Mesocricetus; Norepinephrine--pharmacology--PD; Ouabain--pharmacology --PD; Rubidium--metabolism--ME
CAS Registry No.: 0 (Cyanides); 0 (Iodoacetates); 51-41-2 (Norepinephrine); 630-60-4 (Ouabain); 64-69-7 (Iodoacetic Acid); 7440-17-7 (Rubidium); 7440-23-5 (Sodium); 7440-70-2 (Calcium)
Enzyme No.: EC 3.6.1.- (K ATPase); EC 3.6.1.3 (Adenosinetriphosphatase)
Record Date Created: 19860917
Record Date Completed: 19860917

13/9/3
DIALOG(R)File 155:MEDLINE(R)

07479019 PMID: 3947619
Biochemical and biophysical properties of cytochrome o of Azotobacter vinelandii.
Yang T
Biochimica et biophysica acta (NETHERLANDS) Mar 12 1986, 848 (3) p342-51, ISSN 0006-3002 Journal Code: 0217513
Contract/Grant No.: GM-28308; GM; NIGMS
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Cytochrome o, solubilized from the **membrane** of Azotobacter vinelandii, has been purified to homogeneity as judged by ultracentrifugation and polyacrylamide gel electrophoresis. The

1: Microbiol Immunol. 1983;27(12):985-93. Related Articles, Links

A selective medium for isolation and presumptive identification of the Bacteriodes fragilis group.

Ushijima T, Takahashi M, Tatewaki K, Ozaki Y.

A new selective medium, Bacteroides fragilis ammonium-sulfate gentamicin (BFAG) agar, for isolation and presumptive identification of the Bacteroides fragilis group is presented in this paper. This semisynthetic medium includes 0.2 g of ammonium sulfate, 0.7 g of lactose, 10 mg of gentamicin, 0.1 mg of aminobenzylpenicillin, 60 units of bacitracin, 20 mg of sodium cholate and 1 mg of sodium azide per 100 ml of medium. Stock cultures of the B. fragilis group grew well on this medium. None of the other 126 gram-positive or negative strains belonging to 40 aerobic or 45 anaerobic species tested grew on this medium. Three of the seven specimens in the clinical trials yielded colonies of only the B. fragilis group on BFAG agar plates. Also BFAG agar plates inoculated with human feces and contents of the alimentary tract (stomach, small intestine, cecum and colon) of mice gave rise to colonies of only the B. fragilis group. The high selectivity and good plating efficiency of BFAG agar enabled us to isolate the B. fragilis group rapidly from various clinical specimens.

PMID: 6676629 [PubMed - indexed for MEDLINE]

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DATE: Wednesday, April 06, 2005

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☐	L1	(anoxic or anaerob$ or anoxia).clm. and ((catalase or cata or kata) same (inhibitor or antagonist or blocker or azide or n3 or cyanide)).clm.	0
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END OF SEARCH HISTORY

L4: Entry 16 of 57 File: USPT Jul 14, 1998

DOCUMENT-IDENTIFIER: US 5780239 A
TITLE: Method for the determination of cast in urine

Detailed Description Text (79):
Said analyzer determines presence of casts by comparing absorbance at specified wavelength of the patient's urine and reagent composition complex with that of a standard containing a known concentration of Tamm-Horsfall protein and thereby determining the presence or absence of casts in the patient's urine. This device (method) can use a single, or two-reagent composition in an aqueous medium injected into the reaction cuvette. This device (method) reagent composition of Example 7 can contain compounds to neutralize urine matrix interference and increase urine sample reagent compatibility with the automated analyzer, one or more compounds to remove substances in the urine that cause interference with calorimetric photometry, an activating compound that aids in the coupling reaction between casts, THP and anti-THP Cholesterol oxidase, a surfactant to decrease surface tension and promote mixing on a molecular level and activate the reaction, and a stabilizing agent (such as sodium azide) to prevent color development and stabilize the color indicators or reaction indicators. This device's (method's) wavelength of choice can vary from about 340 to 700 nanometers. Finally, this device (method) for detecting casts in body fluids, comprises contacting a standard or sample of urine, serum, or unknown suspected of containing casts with a reagent composition to detect casts as illustrated for in EXAMPLE 5.

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L4: Entry 20 of 57 File: USPT Feb 27, 1996

DOCUMENT-IDENTIFIER: US 5494812 A
TITLE: Suidatrestin and production thereof

Brief Summary Text (36):
Inhibitor solution (50 .mu.l) and trehalase solution (porcine entrails) 50 .mu.l are mixed, and incubated at 37.degree. C. for 5 min. After addition of 400 .mu.l of 5 mM phosphate buffer (pH 6.3) containing 5 mM trehalose, the resulting mixture is incubated at 37.degree. C. for 15 min. and stopped by adding 1N hydrochloric acid (30 .mu.l). Then, into these 100 ul reactant solution, there are added 0.1M phosphate buffer (pH 6.5) 2.5 ml, 4 mM ABTS 25 .mu.l, 3.5-diaminobenzoic acid 25 .mu.l, and horseradish-peroxidase aqueous solution (1 mg/ml) 10 .mu.l. The resulting mixture is incubated at 37.degree. C. for 5 min., then mixed with glucose-oxidase solution (1 mg/ml) 100 .mu.l and further incubated at 37.degree. C. for 10 min., followed by addition of 1M sodium azide aqueous solution (100 .mu.l) to measure absorbance at 550 nm and obtain an amount of a produced glucose.

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L4: Entry 28 of 57 File: USPT Oct 2, 1990

DOCUMENT-IDENTIFIER: US 4960692 A
TITLE: Assay employing binding pair members on particles and on a filter or membrane

Detailed Description Text (17):
Latex particles of 0.49 um diameter (JSR #U0403) were sensitized with anti-beta-HCG monoclonal antibody and glucose oxidase by the following procedure. Particles which had been washed in 0.01M sodium phosphate buffer pH 7.8 were suspended in the same buffer to a final concentration of about 10%, and 20 mg (0.20 ml) were transferred to a small, glass screw-cap vial. Then, 0.10 ml (0.56 mg protein) of a purified monoclonal anti-beta-HCG antibody (Maritime Chemical Corp.) was added. After incubation at 4.degree. C. overnight, the latex sample was washed once by centrifugation in phosphate buffer, resuspended in 0.10 ml of the same buffer, and 10 mg of solid, purified glucose oxidase (Boehringer Mannheim) added for a total volume of about 0.20 ml. The reaction mixture was incubated for one (1) hour at room temperature, washed twice with 10 mg/ml BSA in PBS by centrifugation and resuspended finally to a concentration of 0.88% (8.8 mg latex/ml) in PBS/BSA containing 0.1% azide.

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L4: Entry 32 of 57 File: USPT Jan 3, 1989

DOCUMENT-IDENTIFIER: US 4795709 A
TITLE: Solvent-induced autolysis of cells

Detailed Description Text (19):
Pichia pastoris NRRL Y-11430 again was produced substantially as described hereinabove. The Pichia pastoris aqueous slurry contained about 130 grams cells per liter, contained about 0.01 weight percent sodium azide, and was adjusted to pH 7.5 as described before. Various amounts of chloroform or methylene chloride were added, and the resulting admixtures were left at room temperature for about four days (96 hours) with occasional shaking. Each sample then was centrifuged at 13,000 rpm for 15 minutes, and the supernatant stored at 4.degree. C. and analyzed thereafter for alcohol oxidase about two days later.

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L4: Entry 36 of 57 File: USPT Oct 28, 1986

DOCUMENT-IDENTIFIER: US 4619898 A
TITLE: Alcohol oxidase from Pichia-type yeasts

Brief Summary Text (38):
At the end of dialysis, the alcohol oxidase is present in the dialysis bag as a crystalline solid. The crystalline alcohol oxidase can be readily separated from the dialysis medium, such as by decanting the liquid in the dialysis bag from the solid crystals. The moist crystals can be further processed as desired for storage. For example, the crystal slurry can be frozen followed by lyophilization to form a dry powder, or can be dissolved in water or more preferably in a phosphate buffer. Stabilizer compounds known to stabilize enzyme solutions against denaturation and loss of enzymatic activity can be added, such as surcrose or glycerol. It is preferable to store the prepared enzyme at temperatures in the range of about 4.degree. C. to 40.degree. C. More preferably, the enzyme is stored at temperatures in the range of about 4.degree. C. to 24.degree. C. Most preferable is storing the enzyme at about 4.degree. C. Only minimal loss of activity has been found to occur when the enzyme is stored at 4.degree. C. in 0.1M Phosphate buffer at pH 7.5, and with 0.02% sodium azide to inhibit microorganism growth. However, my alcohol oxidase can also be stored frozen without significant loss of enzymatic activity.

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L4: Entry 48 of 57 File: USPT Dec 30, 1980

DOCUMENT-IDENTIFIER: US 4242449 A
TITLE: 7-Methoxy cephalosporins and process of producing them

Brief Summary Text (154):
Therefore, in order to obtain selectively the aimed material A (R.sup.1 =Ab), it is desirable to inhibit the catalase activity. Examples of the proper catalase inhibitor are ascorbic acid, 3-amino-1,2,4-triazole, alkali metal azide, etc., and sodium azide is particularly preferable. The inhibitor may be added to the reaction mixture during the conversion of the starting material A (R.sup.1 =Aa) to the aimed material A (R.sup.1 =Ab) or the mycelium may be pretreated by the inhibitor before the mycelium is used in the aforesaid conversion. The amount of sodium azide used for the purpose is about 1-100 mM. Furthermore, the catalase in the aforesaid mycelium can be inactivated by subjecting the mycelium to a heat treatment before use in the aforesaid conversion step. That is, when the aforesaid mycelium is treated at 40.degree.-60.degree. C., preferably at 50.degree. C. for at least 3 hours, it decreases remarkably the catalase activity but, the D-aminoacid oxidase activity remains as it is. The heat treatment may be simply performed to the mycelium in an aqueous solution or a buffer suspension but it is particularly effective to apply the mycelium for the simultaneous aforesaid heat treatment and "activation" reagent treatment. For example, by applying the activation treatment to the mycelium at 50.degree. C. for 4 hours using a solvent, toluene, the inhibition of the catalase activity and the activation of the mycelium can be attained simultaneously.

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L3: Entry 3 of 9 File: USPT Oct 29, 2002

DOCUMENT-IDENTIFIER: US 6472191 B1
**** See image for Certificate of Correction ****
TITLE: DNA FRAGMENT CARRYING TOLUENE MONOOXYGENASE GENE, RECOMBINANT PLASMID, TRANSFORMED MICROORGANISM, METHOD FOR DEGRADING CHLORINATED ALIPHATIC HYDROCARBON COMPOUNDS AND AROMATIC COMPOUNDS, AND METHOD FOR ENVIRONMENTAL REMEDIATION

Brief Summary Text (13):
However, the growth control of the degrading microorganisms is very important for both the expression of the degradation activity on demand and the continuation of degradation. When resting cells are used, it is a problem to be solved that TCE cannot be degraded beyond the amount and period of degradation capacity of the introduced resting cells. In addition, in a large scale treatment, there are further problems that degradation activity will decrease since it takes a long time to prepare resting cells; the treating apparatus must be large in scale; treatment process is complicated; and the cost may be unfavorably high. Accordingly, it has been attempted to introduce a plasmid carrying a DNA fragment containing a gene region encoding oxygenase or hydroxylase into a host microorganism to make the host express the TCE degradation activity constitutively or inducibly using a harmless inducer. For example, there are Pseudomonas mendocina KR-1 (Japanese Patent Application Laid-Open No. 2-503866, Pseudomonas putida KWI-9 (Japanese Patent Application Laid-Open No. 6-105691), Pseudomonas putida BH (Summary of 3rd Conference on Pollution of Ground Water/Soil and Its Protective Countermeasure, p.213 (1994)), and a transformant carrying both a toluene degradation enzyme gene derived from Pseudomonas putida F1 and a biphenyl degradation enzyme gene derived from Pseudomonas pseudoalkaligenes (Japanese Patent Application Laid-Open No. 7-143882). However, the reported TCE degradation activity of the transformants are low, and the advantages of the transformants has not been fully exploited for efficient degradation of TCE, such as the ease of degradation control, freedom in designing recombinant, and no requirements for inducers, far from efficient TCE degradation.

Detailed Description Text (54):
Strain J1 is an aromatic compound-assimilating bacterium which degrades organic halogenated compounds with the participation of oxygenase. In spite of its excellent ability of degrading organic halogenated compounds that it can almost completely degrade about 20 ppm of TCE at a low temperature of 15.degree. C. close to natural environment such as soil, it requires aromatic compounds such as phenol, toluene, and cresol as a degradation inducer. Strain JM1 was obtained by nitrosoguanidine mutagenization of strain J1, and has the same microbiological characteristics as the parental strain J1 except that it can degrade organic halogenated compounds in the absence of aromatic compounds such as phenol, toluene, and cresol as a degradation inducer.

Detailed Description Text (75):
2. Physiological and Biological Properties: Anaerobic growth: Negative Catalase: Positive oxidase: Negative Litmus milk: Alkali Reduction of Nitrate: Negative V-P reaction: Negative pH of V-P medium: pH 7.76 Casein hydrolysis: Negative Gelatin digestion: Negative Starch hydrolysis: Negative DNA hydrolysis: Negative Urea hydrolysis: Negative Tween 20 hydrolysis: Negative Tween 40 hydrolysis: Negative

Tween 60 hydrolysis: Negative Tyrosine hydrolysis: Positive Utilization of organic acids Citric acid: Positive Propionic acid: Positive Acetic acid: Positive Fumaric acid: Positive L-malic acid: Positive Succinic acid: Positive Utilization of inorganic nitrogen: Ammonium salts: positive Nitrates: Positive Indole production: Negative H.sub.2 S production: Negative Pigment production on various media: P agar: Negative F agar: Negative King A agar: Negative King B agar: Negative Growth in the presence of NaCl: 2%: Positive 5%: Negative 7%: Negative Growth pH: 5.0-9.0 Growth temperature: 10.degree. C.-40.degree. C. Growth in the presence of 0.02% sodium azide: negative Growth in the presence of 0.001% lysozyme: positive OF test: negative Production of acid from sugars: Glucose: negative Arabinose: negative Fructose: negative Galactose: negative Maltose: negative Lactose: negative Sucrose: negative Xylose: negative Trehalose: negative Glycerol: negative Mannitol: negative Sorbitol: negative Sorbose: negative Mannose: negative Rhamnose: negative Adonitol: negative Gas production: Glucose: negative Arabinose: negative Xylose: negative Mannitol: negative.

Detailed Description Text (111):
As a result, the combinations of the primers (1) and (3), and (2) and (3) gave the PCR products of about 1.8 kb, and about 1.5 kb, respectively. Then, using these products as the primer in combination with the primer (4) respectively, PCR was carried out. The reaction conditions were the same as the above. As a result, the combinations of the primers ((1)-(3)) and (4), and ((2)-(3)) and (4) gave the PCR products of about 4.8 kb, and about 4.5 kb, respectively. The respective DNA fragments were digested with the restriction enzyme NcoI (Takara Shuzo Co., Ltd.). It was confirmed that the NcoI site in the oxygenase had completely disappeared. These NcoI-digested products were purified using a spin column HR-4000 (Amarsham-Pharmacia) and used for the following ligation reaction.

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APMIS. 2000 Jun;108(6):453-8.

Related Articles, Links

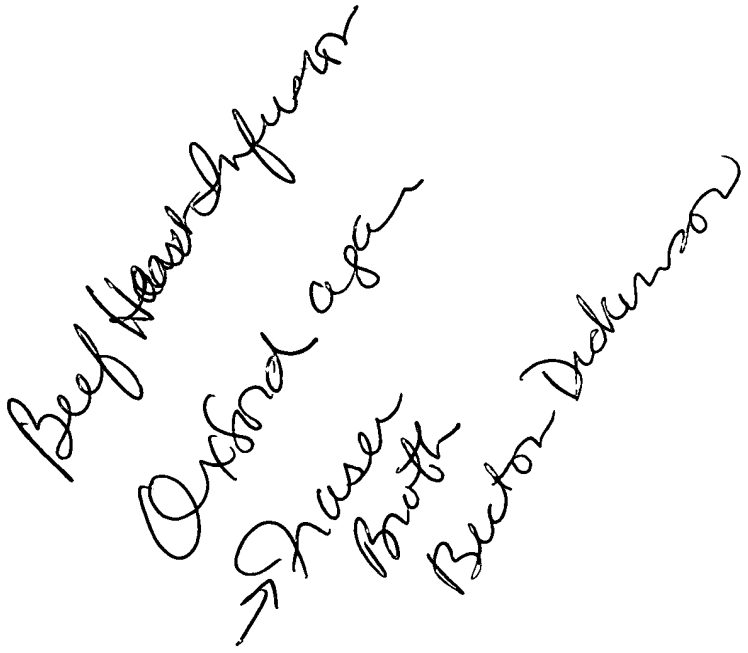
Blood culture bottles for transportation and recovery of anaerobic bacteria from non-blood samples.

Melhus A, Tjernberg I.

Department of Medical Microbiology, Lund University, Malmo University Hospital, Sweden.

Using bacterial suspensions as simulated non-blood specimens, the capacity of three different BacT/ Alert blood culture bottles for the transportation and recovery of anaerobic bacteria with different sensitivity to air was evaluated. To better assess the performance of the BacT/Alert bottles, three other liquid media specially designed for anaerobes were included in the study. Attention was paid to recovery rates in relation to species, initial bacterial concentration, and time needed for detection. Of the BacT/Alert blood culture bottles, the anaerobic FAN bottle yielded the highest recovery rates, but its performance was limited compared with chopped meat broth in tubes. This broth allowed detection of all the tested species within 48 h. Since collection and transportation of anaerobic bacteria are of major importance for a reliable culture result, improvements are necessary.

PMID: 11028809 [PubMed - indexed for MEDLINE



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DATE: Wednesday, April 06, 2005

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☐	L3	select$5 near5 (agar or media or medium or culture or plating)	131783
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☐	L5	(oxygen or o2) near3 (depleted or depletion or removal or scrubbing or removing or removed)	32743
☐	L6	anaerob$5 near2 (conditions or conditioned or environment or growth or growing)	11050
☐	L7	l3 and (l4 or l5 or l6) and (mitochondr$ or membran$)	3540
☐	L8	l3 same(l4 or l5 or l6)	492
☐	L9	L8 and (oxyrase or oxy-rase or oxygenase or membrane-fragments or (membrane near2 fragments))	13
☐	L10	l8 and (azide or cyanide or sodiumazide or sodium-azide or $cyanide)	48
☐	L11	L10 and ((membrane near2 transport) or oxyrase or oxy-rase or oxygenase or membrane-fragments or (membrane near2 fragments))	5

END OF SEARCH HISTORY

Biochemistry (Moscow), Vol. 64, No. 5, 1999, pp. 483-487. Translated from Biokhimiya, Vol. 64, No. 5, 1999, pp. 581-586.

ACCELERATED PUBLICATION

Some Properties of Dissimilatory Nitrate Reductases Lacking Molybdenum and Molybdenum Cofactor

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Abstract—Novel periplasmic and membrane-bound nitrate reductases lacking molybdenum and molybdenum cofactor were isolated from the vanadate-reducing bacterium *Pseudomonas isachenkovii*, and their properties were studied. Both enzymes have some unusual features, i.e., the individual subunits (130-kD subunit of the membrane-bound enzyme and monomeric 55-kD subunit of the periplasmic enzyme) possess their own nitrate reductase activity. In addition, both enzymes are highly thermostable, their temperature optimum being at 70-80°C, which is unexpectedly high for enzymes from mesophilic bacteria. Similarly to conventional molybdenum-containing nitrate reductases, these isolated enzymes are very sensitive to low concentrations of cyanide and azide. During anaerobic cell growth on medium with nitrate and vanadate, nitrate consumption is followed by a period of vanadate dissimilation, and this period is associated with some structural reorganizations of the nitrate reductases.

Key words: nitrate reductase, vanadium-containing enzymes, denitrification

Nitrate reductase (NR) is a key enzyme for nitrate assimilation in eukaryotes [1] and for nitrate assimilation or dissimilation in prokaryotes [2, 3]. The molybdenum dependence of NR was first mentioned in [4] and then confirmed by more than a half-century study of these enzymes [5]. All NRs of bacteria, algae, and plants characterized so far have molybdenum in a pterin molybdenum cofactor (Moco), the universal active site of these enzymes [6, 7]. Molybdenum in NR can be replaced on tungsten, but in this case the enzyme becomes inactive [8]. Vanadium does not replace molybdenum in NR of plants [9] and fungi [10] and unlike molybdenum and tungsten, is not included into molybdenum-free Moco-containing protein isolated from pea seeds [7]. Moreover, vanadium inhibits NR synthesis in plants [11]. All the results obtained so far indicate that molybdenum is unique as a component of the active site of NR. The only data pointing to a possible participation of vanadium in nitrate reduction were obtained by Indian scientists who showed that vanadium (but not molybdenum) stimulated NR activity of a tungsten-tolerant mutant of the cyanobacterium *Nostoc muscorum* [12, 13].

We supposed that vanadium-reducing facultative-anaerobic and facultative-chemolithotrophic *Pseudomonas* bacteria isolated by Lyalikova and Yurkova in the early 90s from vanadium-rich sources—sewage of a plant processing vanadium-containing slags (*Pseudomonas vanadiumreductans*) and ascidia, sea animals capable of accumulating vanadium from sea water and of concentrating it in vanadocytes, special blood chromophores (*Pseudomonas isachenkovii*) [14-16]—could be suitable for a search for vanadium-containing NR.

In the previous work [17] we reported on the isolation of novel membrane-bound and periplasmic NRs lacking molybdenum and Moco from *P. isachenkovii* cells. Also, vanadium was detected in the latter enzyme. The results of further study of these enzymes are discussed in this work.

MATERIALS AND METHODS

In this study we used the following reagents: glycine, acrylamide, ammonium persulfate, TEMED, and triphenyltetrazolium chloride from Sigma (USA); methyl viologen and N-1-naphthylethylenediamine from

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0006-2979/99/6405-0483$22.00

Serva (Germany); Tris and sodium dithionite from Merck (Germany); bis-acrylamide, NaN_3, and KCN from Fluka (Switzerland); yeast extract from Difco (USA). All other reagents were of extra pure grade produced in Russia.

Preparation of biomass. *P. isachenkovii* cells were grown under anaerobic conditions at 30°C for 4-8 days in a 10-liter bottle on modified Postgate medium containing 0.25 g/liter KH_2PO_4, 0.5 g/liter NH_4Cl, 1.0 g/liter $NaNO_3$, 0.5 g/liter $NaVO_3 \cdot 2H_2O$, 1.0 g/liter sodium lactate, and 0.2 g/liter yeast extract. The medium also contained the necessary microelements and trace amounts of $MgSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$. Biomass was harvested after the medium became blue, which indicated that all nitrate had been consumed and the reduction of V^{5+} to V^{4+} had been completed.

Preparation of cell extract. The cells were collected by centrifugation at 4,500*g* for 45 min, washed with 25 mM sodium phosphate buffer (pH 6.8), and disintegrated at 250-280 atm using a French press. To study NR activity as a function of cell growth, the cells were ultrasonicated using a UZDN-1 disintegrator (22 kHz, 3 min, 5°C). The extract was centrifuged at 15,000*g* for 30 min. The supernatant was used as a source of periplasmic NR. The pellet was suspended in 25 mM sodium phosphate buffer containing 5% Triton X-100 for 12 h at 4°C using a magnetic stirrer. Then the suspension was centrifuged at 15,000*g* for 30 min, and the supernatant was used as a source of membrane-bound NR. Homogeneous nitrate reductase preparations were obtained as described in our previous work [17].

Assay of NR activity. Reduced methyl viologen was used as a donor of electrons when assaying NR activity. The reaction mixture contained 100 mM sodium phosphate buffer (pH 6.8), 0.7 mM methyl viologen, 10 mM $NaNO_3$, 1.15 mM sodium dithionite, and 0.01-0.1 ml of the studied preparation. The reaction was initiated by addition of dithionite, performed at 70°C, and stopped by addition of 500 μl of 0.6% sulfanilic acid in 20% HCl and 500 μl of 2 mM N-1-naphthylethylenediamine. Absorbance at 548 nm was measured after 15 min necessary for color development.

To determine nitrate reductase activity in polyacrylamide gel, reaction mixture containing 0.2 M sodium phosphate buffer (pH 6.8), 20 mM KNO_3, 1 mM methyl viologen, and 5 mM sodium dithionite was used. The gel was immersed into this mixture and incubated at 70°C until transparent bands appeared against the blue background of the gel because of oxidation of methyl viologen by nitrate reductase. Then the gel was placed in 0.05% solution of triphenyltetrazolium chloride to fix the staining. Acetic acid (5%) was used for the final fixation of the gel.

Electrophoresis in polyacrylamide gel. Native electrophoresis on 11 × 11-cm plates was performed through a gradient polyacrylamide gel (5-15%) in Tris-HCl buffer (pH 8.8) in the system of Davis [18]. Current was 40 mA in the separating mode. The following standard set of proteins from Serva was used: catalase (240 kD), aldolase (147 kD), BSA (67 kD), ovalbumin (45 kD), and carbonic anhydrase (29 kD). The proteins were stained with silver according to Nesterenko [19].

Protein concentration was determined according to Bradford [20] using bovine serum albumin as a standard.

Nitrate was determined according to Cataldo et al. [21].

Concentration of V^{4+} was determined titrimetrically [22].

RESULTS AND DISCUSSION

The results of determination of nitrate and vanadate (V^{5+}) in the medium during anaerobic growth of *P. isachenkovii* are presented in Fig. 1A. Bacterial cells intensively consumed nitrate during denitrification, usually during the first three days of growth. The second substrate, vanadate, is not dissimilated until nitrate concentration decreases to its lowest value. Calculations performed earlier give an explanation for this [23]. The dynamics of the activity of the membrane-bound and periplasmic NRs during cell culture growth (Fig. 1A) to some extent reflect cell transfer from denitrification to reduction of vanadate: activity decreases together with the disappearance of nitrates from the medium and then, after some time lag perhaps related to "rearrangement" of the enzyme because of the change of the substrate, begins to increase for both enzymes.

As we demonstrated earlier [17], the membrane-bound NR from *P. isachenkovii* has molecular mass 330 kD (subunits 130 and 67 kD) and the periplasmic NR has molecular mass 220 kD and consists of 55-kD monomers. According to the results of determination of NR activity in the gel (Fig. 1B), denitrification involving the membrane-bound NR is first performed by a large subunit of the enzyme (130 kD) which possesses its own NR activity, as we showed in our previous work [17]. As the reduction of vanadate in the cells begins, an oligomeric enzyme complex of the membrane-bound NR with molecular mass 330 kD is detected. The structure of periplasmic NR also changes during cell transfer from denitrification to vanadate reduction (Fig. 1B): denitrification is first performed by an oligomeric enzyme with molecular mass 220 kD, and after nitrate consumption in the medium the enzyme is detected in the form of a

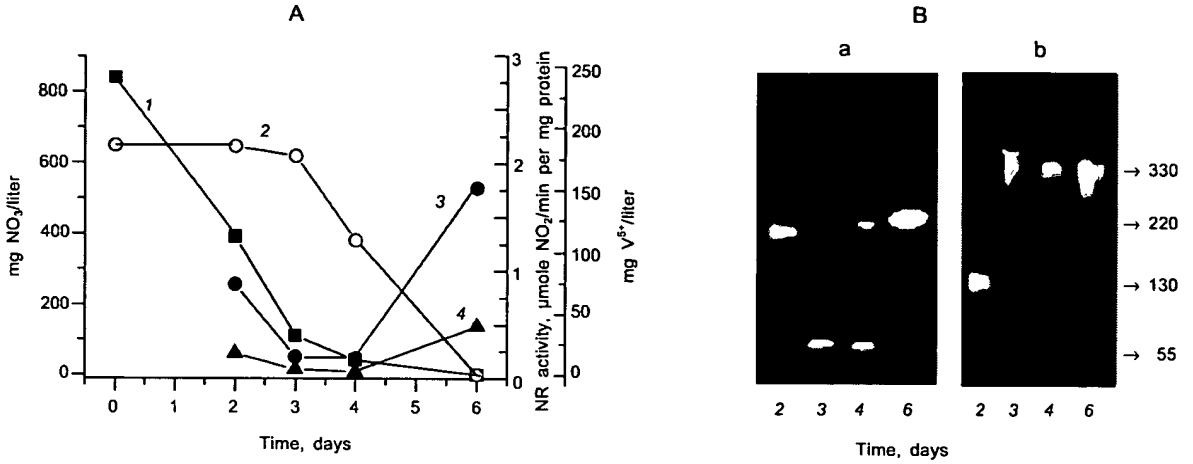

Fig. 1. Reduction of nitrate and vanadate and nitrate reductase activity during anaerobic growth of *P. isachenkovii*. A) Dynamics of reduction of nitrate (*1*) and vanadate (*2*) and activities of the membrane-bound (*3*) and periplasmic (*4*) NRs. B) Change of molecular masses of periplasmic (a) and membrane-bound NRs (b) during culture growth. Molecular masses (on the right, in kD) were determined by native gradient (5-15%) electrophoresis in polyacrylamide gel. The procedure of NR determination in the gel is described in "Materials and Methods".

monomer with molecular mass 55 kD, and on the increase of vanadate-reducing activity of the cells it is detected again as an oligomer with molecular mass 220 kD.

We suppose that changes in the activity and molecular structure of the enzymes described above point to the participation of NR in reduction of not only nitrate, but also vanadate.

Let us discuss some properties of NR isolated from *P. isachenkovii* cells. First, we would like to note the above-mentioned capacity of individual subunits of the membrane-bound (130 kD) and periplasmic (55 kD) NRs to exhibit their own NR activity, as already described in our first work [17]. The high activities of these subunits allows us to detect them in polyacrylamide gel (Fig. 1B). The activity of individual subunits seems to have physiological importance for the cell because subunits rather than oligomeric complexes can be used by the cell for reduction of nitrate and possibly vanadate. Such activity of the enzyme subunits was not detected earlier for any NR described in the literature. Only in several works [3] an active site of NR, Moco, was found to be localized on the large subunits of dissimilatory NR, although the individual catalytic activity of subunits was not demonstrated in these studies.

Judging from some of their properties (molecular mass, temperature optimum, and others), the novel NRs we have isolated from anaerobically growing *P. isachenkovii* cells seem to be synthesized also in aerobically growing cells, but in markedly lower amounts (Fig. 2).

The nitrate reductases we isolated can be synthesized in the presence of high concentrations of ammonium in the medium; in this respect they differ from the molybdenum-containing dissimilatory NR.

Activities of both NRs are maximal at pH 6.8-7.0, although rather high activity was detected in the wider range of pH from 6.0 to 8.0 (Fig. 3a). The studied NRs

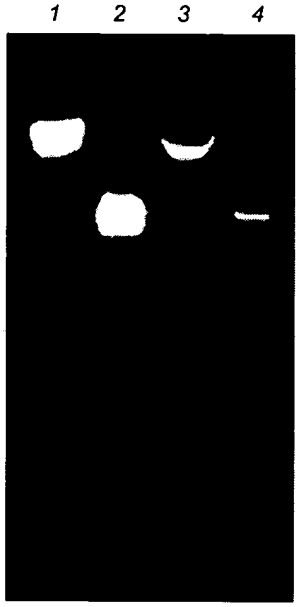

Fig. 2. Determination of nitrate reductase activity of extracts of *P. isachenkovii* cells grown under anaerobic (membrane bound (*1*) and periplasmic (*2*) NRs) and aerobic (membrane-bound (*3*) and periplasmic (*4*) NRs) conditions. NR samples from aerobic cells contained 50 μg of protein and that from anaerobic cells contained 5 μg of protein.

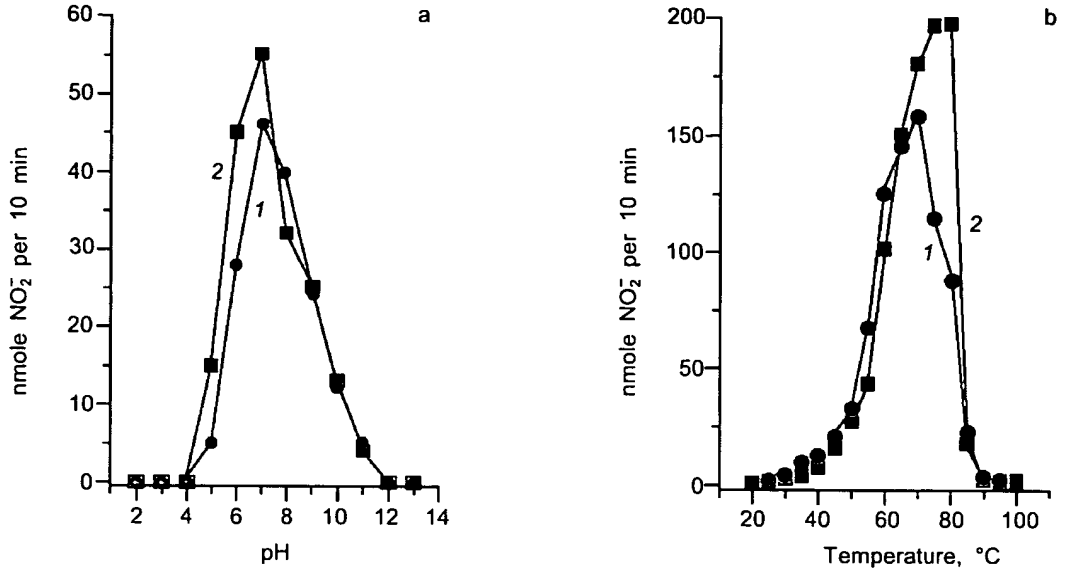

Fig. 3. pH (a) and temperature (b) optima for nitrate reductase activities: *1*) membrane-bound NR; *2*) periplasmic NR. Buffers used in (a): 0.2 M glycine-HCl, pH 2-3; 0.2 M Na-AcOH, pH 4-5; 0.2 M sodium phosphate, pH 6-8; 0.2 M glycine-NaOH, pH 9-10; 0.2 M Na_2HPO_4-NaOH, pH 11; 0.2 M KCl-NaOH, pH 12-13. In (b) the standard reaction mixture for determination of nitrate reductase activity was used. In both (a) and (b) data for homogeneous preparations of both NRs are shown.

have temperature optimum at 70-80°C (Fig. 3b); this is unexpectedly high for enzymes from mesophilic bacteria. The activity of both enzymes remained unchanged at 50°C for 2 h, whereas incubation at 70°C for 40 min resulted in the loss of 80% of the NR activity. High thermal stability is also typical of vanadium-containing haloperoxidases [24-26].

The isolated NRs were inhibited by low concentrations of cyanide or azide; this feature is typical of the conventional molybdenum-containing dissimilatory NRs. $I_{0.5}$ was 70 and 60 μM for the membrane-bound and 40 and 20 μM for the periplasmic NR, respectively (Fig. 4). Inhibition by cyanide and azide suggests the presence of a metal atom in the active site.

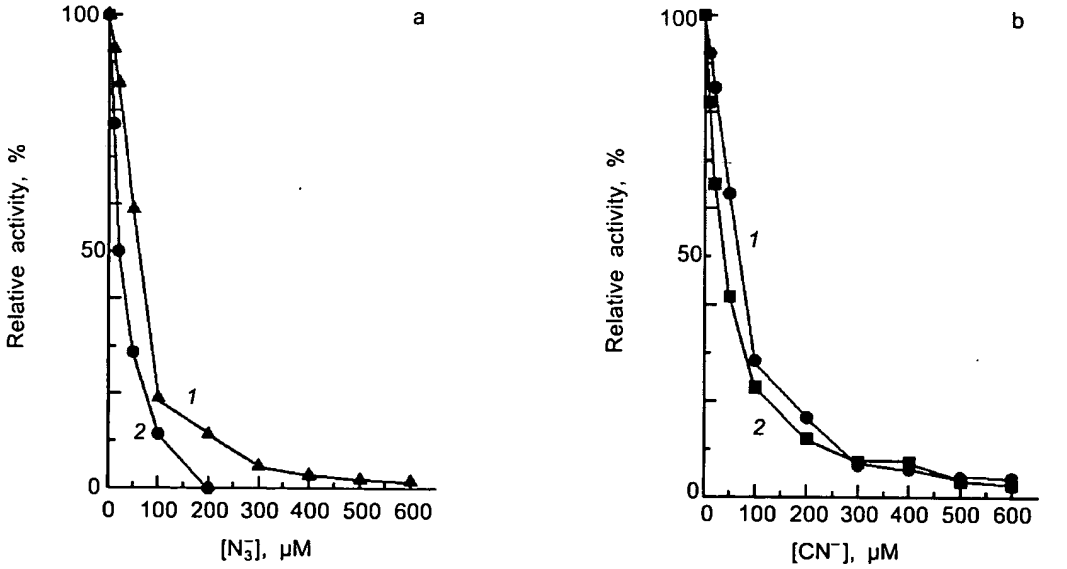

Fig. 4. Effect of azide (a) and cyanide (b) on the activities of nitrate reductases: *1*) membrane-bound NR; *2*) periplasmic NR. Homogeneous NR preparations of both types were used in the experiments.

Thus, two novel NRs lacking molybdenum and Moco (one of them containing vanadium) were isolated from *P. isachenkovii*, and this resembles the situation in the study of nitrogenase which was done in the early 80s. Then, first a vanadium-containing nitrogenase and subsequently an iron-containing nitrogenase lacking molybdenum and vanadium were discovered [27-29]. Along with the alternative nitrogenase, vanadium was also detected in some haloperoxidases catalyzing oxidation of halogen ions by hydrogen peroxide (bromo-, chloro-, and iodoperoxidases), as mentioned above. Several haloperoxidases have structures similar to that of the periplasmic NR isolated by us [24]. Except for the alternative nitrogenase and the haloperoxidases, we do not know of any other vanadium-containing enzymes being reported [30, 31].

The isolation of a novel NR lacking molybdenum and Moco from vanadium-reducing bacteria raises some questions: are these alternative enzymes widespread, and under what conditions and in which bacteria are they synthesized? Concerning the vanadium-containing NR, it is necessary first to remember that the content of vanadium in the biosphere is 20 times greater than that of molybdenum [32]; perhaps in regions with decreased molybdenum content in the soil, molybdenum-deficient plants can synthesize the alternative vanadium-containing NR. The appearance of the alternative NR is especially possible in bacteria growing at high concentrations of toxic heavy metals, when synthesis of the normal molybdenum-containing NR is suppressed. This seems to occur in cells of the above-mentioned tungsten-tolerant mutant of the cyanobacterium *Nostoc muscorum* [12, 13] and an *E. coli* mutant tolerant to high concentrations of tellurite and selenate [33]. Ivanova's studies on the nitrate reductase activity of plant peroxidases published in the 70s and 80s should be also mentioned [34-37]. These enzymes use superoxide radicals for reduction of nitrates and can to some extent replace the normal NR in plant growth under stress conditions. These works as well as our isolation of novel nitrate reductases demonstrate that alternative pathways of nitrate reduction could be used by living organisms.

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Arch Inst Pasteur Alger. 1992;58:161-8. Related Articles, Links

[Efficiency of inhibitors (phenylethanol, nalidixic acid, sodium azide)in the isolation of strictly anaerobic bacteria from a polymicrobial specimen]

[Article in French]

Merad AS, Ghemati M.

Laboratoire des Anaerobies, Institut Pasteur d'Algerie, Alger.

The efficacy of inhibitors (PEA, Ac Nal, Az de Na) in the isolation of strict anaerobes in polymicrobial sample. The efficacy of three inhibitors to select strict anaerobic bacteria in the polymicrobial sample had been studied. First step: The most frequent anaerobes encountered in the infections are isolated in the agar Columbia containing the different inhibitors. This step allowed us to check the inhibition of the germ we have to isolate. Next step: polymicrobial mixtures were made. The composition of which is very similar to the samples we receive in the laboratory. The swarming Proteus is the facultative anaerobic germ which gives us difficulties when isolating strict anaerobic bacteria. Then, the different mixtures were isolated separately in the agar in which the inhibitors were added. The plates containing Azide of Na and PEA gave us the best results.

PMID: 1285025 [PubMed - indexed for MEDLINE]

Pathology. 1986 Jan;18(1):141-4. Related Articles, Links

A comparison of selective media for the isolation of anaerobic bacteria from clinical material.

Downes J, Stern L, Andrew JH.

Clinical specimens submitted for anaerobic culture to a Melbourne teaching hospital microbiology laboratory were plated onto 3 types of selective media, to determine which would allow the optimal recovery of anaerobic organisms. The 3 media employed were kanamycin agar (KA), neomycin agar (NA) and nalidixic acid-Tween 80 agar (NAT). The highest isolation rate was achieved on NAT, 89% of the total of all anaerobes isolated being recovered on this medium. A recovery rate of 69% was achieved using NA, while use of KA allowed the isolation of only 56% of all strains. The major difference between 3 media was in the recovery of anaerobic Gram-positive cocci, which accounted for 40% of the total isolates on NAT, 25% on NA, and only 11% on KA. The NAT was also more successful in the isolation of Fusobacterium and Veillonella species. The NAT medium failed, however, to recover Clostridium spp. that were isolated on both NA and KA. There was no significant difference between the 3 media in regard to the recovery of Bacteroides spp.

Publication Types:
- Case Reports

Bull Tokyo Dent Coll. 1998 May;39(2):103-7. Related Articles, Links

Semiquantitative bacteriology of closed odontogenic abscesses.

Sakamoto H, Kato H, Sato T, Sasaki J.

Department of Oral Surgery, Tokai University, School of Medicine, Kanagawa, Japan.

Pus samples from twenty-three dentoalveolar abscesses were collected by needle aspiration and examined by direct inoculation technique using 6 different aerobic and anaerobic agar plates. For aerobic culture, sheep blood agar and chocolate agar (Kyokuto Pharmaceutical Co., Tokyo, Japan) were used. For anaerobic culture, four different media, 1) brucella HK agar with hemolyzed rabbit blood and defibrillated sheep blood, 2) paramomycin-vancomycin brucella HK agar with hemolyzed rabbit blood, 3) phenylethyl alcohol brucella HK agar with hemolyzed rabbit blood, 4) bacteroides bile esculin agar (Kyokuto Pharmaceutical Co., Tokyo, Japan) were prepared in an anaerobic jar prior to inoculation. The aerobic agar plates were incubated for 24 h at 37 degrees C, and the anaerobic plates at least 48 h at 37 degrees C in anaerobic jars. From 23 closed odontogenic abscess samples, a total of 112 bacterial strains were isolated; 81 strains (72.3%) were strict anaerobes, and 31 strains (27.7%) were aerobes. The mean number of bacterial strains per positive sample was 4.86. Oral Streptococci, Prevotella, Fusobacterium, Peptostreptococcus and Veillonella were common isolates. The combination of Oral Streptococci and Prevotella was found in 11 patients (47.8%), and that of Prevotella and Peptostreptococcus in 12 patients (52.2%). The present study demonstrated that closed odontogenic abscesses are polymicrobial infections by aerobes and anaerobes. Application of a direct inoculation technique for bacterial culture made it possible to isolate more anaerobes than our commonly used technique using transport medium and to delineate the semiquantitative bacteriology of closed odontogenic abscess.

PMID: 9667143 [PubMed - indexed for MEDLINE]

Bull Tokyo Dent Coll. 1998 May;39(2):103-7. Related Articles, Links

Semiquantitative bacteriology of closed odontogenic abscesses.

Sakamoto H, Kato H, Sato T, Sasaki J.

Department of Oral Surgery, Tokai University, School of Medicine, Kanagawa, Japan.

Pus samples from twenty-three dentoalveolar abscesses were collected by needle aspiration and examined by direct inoculation technique using 6 different aerobic and anaerobic agar plates. For aerobic culture, sheep blood agar and chocolate agar (Kyokuto Pharmaceutical Co., Tokyo, Japan) were used. For anaerobic culture, four different media, 1) brucella HK agar with hemolyzed rabbit blood and defibrillated sheep blood, 2) paramomycin-vancomycin brucella HK agar with hemolyzed rabbit blood, 3) phenylethyl alcohol brucella HK agar with hemolyzed rabbit blood, 4) bacteroides bile esculin agar (Kyokuto Pharmaceutical Co., Tokyo, Japan) were prepared in an anaerobic jar prior to inoculation. The aerobic agar plates were incubated for 24 h at 37 degrees C, and the anaerobic plates at least 48 h at 37 degrees C in anaerobic jars. From 23 closed odontogenic abscess samples, a total of 112 bacterial strains were isolated; 81 strains (72.3%) were strict anaerobes, and 31 strains (27.7%) were aerobes. The mean number of bacterial strains per positive sample was 4.86. Oral Streptococci, Prevotella, Fusobacterium, Peptostreptococcus and Veillonella were common isolates. The combination of Oral Streptococci and Prevotella was found in 11 patients (47.8%), and that of Prevotella and Peptostreptococcus in 12 patients (52.2%). The present study demonstrated that closed odontogenic abscesses are polymicrobial infections by aerobes and anaerobes. Application of a direct inoculation technique for bacterial culture made it possible to isolate more anaerobes than our commonly used technique using transport medium and to delineate the semiquantitative bacteriology of closed odontogenic abscess.

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Scand J Urol Nephrol Suppl. 1984;86:117-24. Related Articles, Links

Anaerobic curved rods in genital samples of women. Performance of different selective media, comparison of detection by microscopy and culture studies, and recovery from different sampling sites.

Holst E, Hofmann H, Mardh PA.

Anaerobic curved rods (CR) frequently occur in the vaginal flora of women with non-specific vaginitis, more recently referred to as bacterial vaginosis (BV). The reasons for difficulties in culturing CR include their anaerobic nature, slow rate of growth and presence in a highly mixed flora. The present study concerns the efficiency of three culture media--blood agar, a gonococcal medium, and Columbia agar--for recovery of CR. The possibility of improving selectivity by adding various antibiotics (e.g. nalidixic acid, colistin and tinidazole) to the media was also explored. The MICs for 157 CR strains and for 80 strains of anaerobic or facultatively anaerobic bacteria isolated from vaginal samples from women with BV were therefore determined. Columbia agar containing 1 micrograms/ml tinidazole in combination with either colistin (10 micrograms/ml) or nalidixic acid (15 micrograms/ml) proved the most efficient medium for recovery of CR. These antibiotic combinations effectively suppressed growth of Gardnerella vaginalis, anaerobic cocci and species of Bacteroides.--In 291 women, comparison was made of the detection of CR morphotype bacteria in methylene-blue-stained smears and the results of vaginal cultures for CR. A long variant (2-4 micron) was found in 42% by direct microscopy and 43% by culture. A short variant (approx 1 micron), which, with two exceptions, was always concomitant with the long variant, was demonstrable in only 3% of vaginal smears and in 14% of the cultures.--Vaginal samples were approximately four times more often culture-positive for CR than were cervical samples from the same women.

PMID: 6399403 [PubMed - indexed for MEDLINE]

Zentralbl Bakteriol [Orig A]. 1977 Nov;239(3):375-8. Related Articles, Links

Media supplemented with nalidixic acid for the isolation of gram negative anaerobic bacteria.

Nadaud M.

The minimum inhibitory concentrations (MIC) of nalidixic acid were determined for thirty-four strains of Clostridium sp. by an agar dilution technique. The MIC range of Clostridium perfringens ran from 0.03 microgram/ml to 64 microgram/ml, according to a bimodal distribution. One half of the strains was inhibited by 0.25 microgram/ml. 64 microgram/ml was able to inhibit all the strains tested. The author suggests a systematic utilization of media with and without 40 microgram/ml nalidixic acid. These two media were parallely inoculated with clinical specimens where anaerobic bacteria were suspected, and allowed the isolation of gram negative anaerobes.

PMID: 203144 [PubMed - indexed for MEDLI

Taehan Chikkwa Uisa Hyophoe Chi. 1985 Feb;23(2):121-7. Related Articles, Links

[Isolation of anaerobic bacteria from oral pyogenic infections. Effects of strict anaerobic procedure and culture media]

[Article in Korean]

Jang BS, Kim CW, Nam IW, Min PI, Hwang SM, Choe SJ.

PMID: 3886811 [PubMed - indexed for MEDLINE]

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Use of Presumpto Plates To Identify Anaerobic Bacteria

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Identification of anaerobic bacteria requires special media and growth conditions that contribute to a higher cost per identification than that for aerobic isolates. Newer rapid methods streamline the identification process, but confirmation to the species level is often difficult. The Presumpto Plate method for the identification of commonly encountered anaerobes consists of three quadrant plates, each containing four conventional media, that result in the generation of 21 test parameters: growth on Lombard-Dowell medium; production of indole, indole derivative, catalase, lecithinase, and lipase; proteolysis of milk, H_2S, and esculin; growth on 20% bile; precipitate on bile; DNase, glucose, casein, starch, and gelatin hydrolysis; and fermentation of lactose, mannitol, and rhamnose. Identification charts were developed by using the results from 2,300 anaerobic isolates. Because conventional media were used, there was a high degree of agreement between the Presumpto Plate method and the reference method when testing commonly encountered anaerobes. The Presumpto Plate method is as accurate as commercially available enzyme systems for the identification of many anaerobic species but is less expensive to perform.

In 1975, the Anaerobic Laboratory at the Center for Disease Control (CDC) began work to develop what eventually became three quadrant plates: Presumpto Plates I, II, and III, containing various differential media prepared from Lombard-Dowell (LD) agar (4). These three plates provided 21 tests for identifying anaerobic bacteria. Presumpto Plate I was initially developed to assist in identifying the gram-negative anaerobes *Bacteroides* and *Fusobacterium* spp. (3) (see Table 4). Plates II and III provided additional tests for identifying of gram-positive isolates (7, 8). LD agar supports the growth of a wide variety of anaerobic bacteria, including fastidious strains (2). Although used for many years at CDC and other sites, the accuracy of the Presumpto Plate method of anaerobic identification has not been reviewed. The purpose of this study was to evaluate the Presumpto Plate method and provide information that may be helpful in designing an abbreviated and cost-effective reference procedure for the identification of commonly encountered anaerobic bacteria.

MATERIALS AND METHODS

Organisms. More than 2,300 strains of anaerobic bacteria were tested to generate the data shown in the tables.

Definitive identification was based on CDC conventional reference procedures (1, 5, 6). The culture and biochemical characteristics of the anaerobic bacteria in this study were derived from the examination of human clinical isolates submitted to CDC for identification or confirmation from federal, state, or city laboratories. Additional data were derived from the examination of isolates from animals and from reference strains obtained from culture collections or individual investigators.

Inoculation procedure. All cultures were plated on anaerobic blood agar and checked for purity. Isolated colonies were inoculated into either thioglycolate broth or LD broth and on anaerobic blood agar plates and incubated at 35°C in an anaerobic glove box containing an atmosphere of 5% CO_2, 10% H_2, and 85% N_2 for 12 to 48 h. The inoculum for the Presumpto Plates was prepared with either an enriched thioglycolate broth culture or a cell suspension in LD broth equivalent to the density of a number 5 McFarland standard prepared from colonies growing on an anaerobic blood agar plate. A swab was dipped in the suspension, and the excess fluid was removed by pressing the swab gently against the wall of the tube. Each quadrant of the Presumpto Plate was inoculated with a single streak beginning near the center of the plate and streaking outward toward the periphery. After a quadrant was inoculated, the swab was recharged with inoculum and the next quadrant inoculated. This process was repeated until all three Presumpto Plates were inoculated. A sterile, 6-mm-diameter paper disk was placed over the streak in the LD agar quadrant of Presumpto Plate I for indole testing. Within 15 min after inoculation, the plates were placed in an anaerobic glove box and incubated at 35°C for 24 to 48 h. After incubation, the plates were removed from the glove box, the necessary test reagents were added to the proper quadrant, and the results were recorded. Reactions were determined as shown in Table 1. In this study commercially prepared Presumpto Plates were used (Carr Scarborough Microbiologicals Inc., Stone Mountain, Ga.). For laboratories that wish to prepare their own media, the preparation procedures are given below.

Preparation procedures for Presumpto Plates. Presumpto Plate I quadrants contain 5.0 ml each of LD agar, LD-esculin agar, LD-bile agar, and LD-egg yolk agar in a plastic petri dish (15 by 100 mm).

LD agar contains the following: Trypticase (Becton Dickinson Microbiology Systems, Cockeysville, Md.), 5.0 g; yeast extract (Difco Laboratories, Detroit, Mich.), 5.0 g; sodium chloride, 2.5 g; sodium sulfite, 0.1 g; L-tryptophan, 0.2 g; vitamin K_1 (3-phytylmenadione), 0.01 g; agar, 20.0 g; distilled water, 1,000.0 ml; L-cystine, 0.4 g; and hemin, 0.01 g. Dissolve the L-cystine and hemin in 1 N sodium hydroxide before adding them to the medium. Add vitamin K_1 from a 1% stock solution prepared in absolute ethanol and autoclaved at 121°C for 15 min with a final pH adjusted to 7.4.

Prepare LD-esculin agar as described above, except omit the sodium sulfite from the LD agar and substitute 1.0 g of esculin and 0.5 g of ferric citrate per liter before autoclaving.

For LD-bile agar, LD agar is supplemented with 20 g of oxgall (Difco) and 1 g of glucose per liter before it is autoclaved and dispensed into the third quadrant of the Presumpto Plate I.

For LD-egg yolk agar, egg yolk agar base is prepared with Trypticase (Becton Dickinson), 5.0 g; yeast extract (Difco), 5.0 g; sodium chloride, 2.5 g; sodium sulfite, 0.1 g; L-tryptophan, 0.2 g; L-cystine, 0.4 g; hemin, 10.0 mg; vitamin K_1, 10.0 mg; D-glucose, 2.0 g; Na_2HPO_4, 5.0 g; $MgSO_4$ (5% aqueous solution), 0.2 ml; agar, 20.0 g; and distilled water, 900 ml. Mix the ingredients for the base and dissolve them by heating. When all components are in suspension, adjust the pH to 7.4 and autoclave the medium for 15 min at 121°C. After autoclaving, place the flask in a water bath and cool it to 50 to 60°C. Warm the egg yolk suspension (100 ml) (Difco) in a water bath to 55°C, add it to the cooled base, and then dispense it into the remaining quadrant of the plastic plate.

The LD Presumpto Plate II contains LD-glucose agar, LD-starch agar, LD-milk agar, and LD-DNA agar.

For LD-glucose agar, LD agar base is supplemented with 6.0 g of D-glucose per liter and 2 ml of 1% bromthymol blue before 5 ml is autoclaved and dispensed into a quadrant of the plastic plate.

For LD-starch agar, LD agar base is supplemented with 5.0 g of soluble starch (Difco) per liter before it is autoclaved and dispensed into the second quadrant of the plate.

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TABLE 1. Presumpto Plate reactions

Test	Quadrant or Reagent(s)	Interpretation[a]
Presumpto I		
Indole	LD agar, indole reagent	Pos, blue to blue-green; indole derivative, lavender; neg, colorless or pink
Catalase	LD agar, H_2O_2	Pos, bubbles; neg, no bubbles
Lecithinase	Egg yolk agar	Pos, precipitate around growth; neg, no precipitate
Lipase	Egg yolk agar	Pos, iridescent sheen or pearly layer on colonies and surrounding medium; neg, no pearly layer or sheen
Proteolysis	Egg yolk agar	Pos, clearing of medium around growth; neg, no clearing of medium
H_2S	Esculin agar	Pos, blackening of medium; neg, no blackening
Esculin hydrolysis	Esculin agar, Wood's lamp	Pos, red-brown in agar after 5-min exposure to air plus no blue fluorescence; neg, no brown color with bright blue fluorescence
Catalase	Esculin agar, H_2O_2	Pos, bubbles; neg, no bubbles
Growth	Bile agar	I, inhibited vs that on LD agar; E, equal to that on LD agar
Precipitate	Bile agar	Precipitate present in agar
Presumpto II		
DNase	DNA agar	Pos, pink or reddish in agar; neg, no color change
Glucose	Glucose agar	Pos, yellow in medium; neg, blue or blue-green
Casein hydrolysis	Milk agar	Pos, clearing of agar around growth; neg, no clearing of agar
Starch hydrolysis	Starch agar, Gram's iodine	Pos, clear zone around growth; neg, brown or black around growth
Presumpto III		
Lactose fermentation	Lactose agar, bromthymol blue	Pos, yellow; neg, blue-green
Mannitol fermentation	Mannitol agar, bromthymol blue	Pos, yellow; neg, blue-green
Rhamnose fermentation	Rhamnose agar, bromthymol blue	Pos, yellow; neg, blue-green
Gelatin hydrolysis	Gelatin agar, Frazier's reagent	Pos, clear zone around growth; neg, opaque agar, no clearing

[a] Pos, positive; neg, negative.

For LD-milk agar, LD base is supplemented with 50.0 g of powdered skim milk (Difco or Becton Dickinson) per liter before being autoclaved and dispensed.

For LD-DNA agar, LD base is supplemented with 1.25 g of DNA (polymerized) and 25 ml of toluidine blue O (0.25% aqueous solution) per liter, autoclaved, and dispensed into the fourth quadrant of the plastic plate.

LD Presumpto Plate III contains LD-mannitol agar, LD-lactose agar, LD-rhamnose agar, and LD-gelatin agar. Prepare the LD-mannitol agar, LD-lactose agar, and LD-rhamnose agar by supplementing LD base with 6.0 g of the respective carbohydrates per liter before autoclaving and dispensing them into the sections of the quadrant plate. Prepare gelatin agar by adding 4.0 g of gelatin and 1.0 g of D-glucose per liter to the LD agar base before autoclaving and dispensing it into the last quadrant of the Presumpto Plate III. This plate is useful for differentiating gram-negative as well as gram-positive anaerobic bacilli. The shelf life for sealed sleeves of media of Presumpto Plates is 3 to 5 weeks.

Reagents. Prepare test reagents for the Presumpto Plate I as follows. For indole reagent, dissolve 1 g of paradimethylamino-cinnamaldehyde (Aldrich Chemical Co., Milwaukee, Wis.) and dilute it to 100 ml with dilute HCl (10 ml of concentrated HCl plus 90 ml of distilled water). Store it in a dark bottle, and keep it refrigerated when not in use. For catalase reagent, 3% hydrogen peroxide is used and should also be stored refrigerated.

Reagents for the Presumpto Plate II are Gram's iodine, used to detect hydrolysis of starch, and bromthymol blue (1%), which is added dropwise to the carbohydrate quadrant of Presumpto Plate III because in an anaerobic atmosphere the indicator may be reduced to colorless, thus preventing accurate interpretation of the reaction. Frazier's solution (mercuric chloride, 15.0 g; concentrated HCl, 20.0 ml; and distilled water, 100.0 ml) is used to demonstrate hydrolysis of gelatin.

Interpretation. Presumpto Plate I. A summary of the expected Presumpto Plate I reactions is presented in Table 1. To the paper disk add 2 drops of the indole reagent and observe for the development of blue or bluish-green color on the disk within 30 s, which indicates a positive reaction for indole. The absence of color or the development of another color (pink or red) is considered a negative result. A lavender to violet shade indicates a positive reaction for indole derivative, a significant finding for some clostridia.

Lecithinase production on LD-egg yolk agar is indicated by a zone of insoluble precipitate in the medium surrounding the bacterial growth. In the same quadrant, lipase production is indicated by an iridescent sheen or pearly layer on the surface of the bacterial colonies and on the medium near the bacterial growth. Proteolysis is indicated by a clearing of the medium in the vicinity of bacterial growth.

Before removing the Presumpto Plate I from the anaerobic atmosphere, observe the esculin agar for blackening of the colonies, which indicates H_2S production on the esculin agar that will dissipate rapidly after exposure to air. Observe for esculin hydrolysis after exposing the plate to the air for at least 5 min. A reddish-brown to dark brown color developing in the esculin agar surrounding the bacterial growth indicates a positive result. Examine the esculin agar quadrant under a Wood's lamp for further evidence of esculin hydrolysis. Intact esculin agar exhibits a bright-blue fluorescence under the UV light, which is lost if the esculin is hydrolyzed. After the plates have been exposed to air for 30 min, test the colonies on esculin agar for catalase production by adding a few drops of fresh 3% hydrogen peroxide to the growth. Sustained bubbling indicates a positive reaction for catalase.

Compare the degree of bacterial growth on the LD-bile agar with that on the LD agar. If the degree of growth on the LD-bile agar is inhibited and therefore less than that on the LD agar, record an "I." If the growth is equal to or greater than the growth on the LD-agar control, record an "E." Note the presence or absence of a precipitate in this quadrant.

Presumpto Plate II. A positive DNase test is indicated by a pink to reddish color around the bacterial growth on the DNA quadrant.

Glucose fermentation is indicated when the medium in this quadrant exhibits a yellow color around the bacterial growth. Because the indicator may be reduced by the anaerobic atmosphere rather than by the organism, bromthymol blue indicator may be added dropwise to the surface of the medium if better contrast is needed.

LD-milk agar demonstrates casein hydrolysis. A positive test is indicated by a clearing of the milk around the growth on the quadrant.

Hydrolysis of starch is determined by flooding the LD-starch quadrant with Gram's iodine and observing for a clear zone around the growth. A brownish color indicates no hydrolysis of starch and a negative reaction.

TABLE 2. Quality control for Presumpto Plates

Medium	Organism[a]	Expected reaction(s)[b]
LD agar	*Bacteroides fragilis*	Moderate growth, indole −, catalase +
	Bacteroides thetaiotaomicron	Moderate growth, indole +, catalase −
LD-esculin	*B. fragilis*	Esculin +, H_2S −, catalase +
	Porphyromonas asaccharolytica	Esculin −, H_2S −, catalase −
	Fusobacterium mortiferum	Esculin +, H_2S +, catalase −
LD-bile	*B. fragilis*	Growth, E; precipitate +
	Fusobacterium necrophorum	Growth, I; precipitate −
LD-egg yolk	*B. fragilis*	Lecithinase −, lipase −, proteolysis −
	Clostridium sporogenes	Lecithinase −, lipase +, proteolysis +
	Clostridium novyi A	Lecithinase +, lipase +, proteolysis −
LD-glucose	*Clostridium innocuum*	+ (yellow)
	Clostridium tetani	− (blue-green)
LD-milk	*C. sporogenes*	+ (digestion)
	Clostridium perfringens	− (no digestion)
LD-DNA	*C. perfringens*	+ (pink)
	C. sporogenes	− (no color change)
LD-starch	*C. perfringens*	+ (clearing with iodine)
	C. sporogenes	− (no clearing)
LD-lactose	*Bacteroides vulgatus*	+ (yellow)
LD-rhamnose	*Clostridium subterminale*	− (blue-green)
LD-mannitol	*Propionibacterium acnes*	+ (yellow)
	C. subterminale	− (blue-green)
LD-gelatin	*C. sporogenes*	+ (clearing with reagent)
	B. fragilis	− (no clearing)

[a] Any strain that would provide the expected reaction(s) is adequate and may include American Type Culture Collection strains or fresh isolates.
[b] −, negative; +, positive; E and I, see Table 1 for definitions.

Presumpto Plate III. The LD-lactose agar, LD-mannitol agar, and LD-rhamnose agar are interpreted like the LD-glucose agar. To test for gelatin hydrolysis, flood the quadrant with acidified mercuric chloride solution (Frazier's reagent), which binds with intact gelatin, causing the medium to appear opaque. A clear zone around the growth is a positive test for gelatin hydrolysis.

Quality control procedures for all three plates are listed in Table 2.

RESULTS AND DISCUSSION

The Presumpto Plate system is a three-plate method developed for the identification of anaerobes that provides 21 observations or reactions. We have tested and recorded results for over 2,300 anaerobic isolates from which identification charts have been produced (Table 3). This method uses conventional anaerobic media with minimum costs to arrive at an accurate identification of even fastidious anaerobes.

Presumpto Plate I is primarily used to separate *Bacteroides* spp. and *Fusobacterium* spp. (Table 4), but it can also be useful in the identification of anaerobic cocci, non-spore-forming gram-positive bacilli, and *Clostridium* spp.

With the addition of Presumpto Plate II, better separation and identification of the clostridia, anaerobic cocci, non-spore-forming gram-positive bacilli, and anaerobic non-spore-forming gram-negative bacilli are achieved. Presumpto Plate III, which includes gelatin agar, helps in the identification of additional anaerobic species, such as *Actinomyces pyogenes* and *Bacteroides levii*.

The production of lecithinase and lipase with proteolytic activity on gelatin is a key characteristic for separating the gram-positive spore-forming rods from other groups. Catalase production and the fermentation of mannitol are helpful in separating some of the gram-positive non-spore-forming rods.

When used in conjunction with other tests, Presumpto Plates provide a useful and convenient method for the identification of anaerobic bacteria at a reasonable cost. The cost of Presumpto Plates is about $1.41 per plate, or $4.23 when all three are used. For two popular commercial enzyme systems the cost is between $5.00 and $5.20 per panel. If an isolate is common, only Presumpto Plate I may be needed to identify it. The number of plates used to identify any isolate may vary. For laboratories that identify anaerobic bacteria, the Presumpto Plate method may offer a substantial cost savings in materials. The additional cost of quality control for Presumpto Plates would have to be considered.

These plates are not considered rapid methods as are the newer enzyme systems, but they represent a potentially more accurate means of identification because they are based on conventional procedures whose interpretation may be more familiar to microbiologists accustomed to reading pH-based tests and other classical reactions. For a skilled microbiologist,

TABLE 3. Identification chart

Species	No. of strains	% Positive reactions on differential media from: CDC Presumpto Plate I: Indole	Indole derivative	Esculin	H_2S-esculin	Catalase-LD	Catalase-esculin	Lecithinase	Lipase	Egg yolk agar proteolysis	Bile agar: E growth	Bile agar: I growth	Bile agar: No growth	Bile agar: Precipitate	CDC Presumpto Plate II: Glucose	Starch hydrolysis	Protcolysis of milk	DNase	DNA clearing	Catalase-milk	CDC Presumpto Plate III: Gelatin	Mannitol	Lactose	Rhamnose	Pink with HgCl
Actinomyces bovis	3	0	0	100	0	0	0	0	0	0	0	67	33	0	100	33	0	0	33	0	0	0	67	0	0
Actinomyces hordeovulneris	4	0	0	50	0	100	75	0	0	0	0	25	75	0	75	0	0	0	50	100	0	0	25	0	0
Actinomyces howellii	1	0	0	100	0	100	100	0	0	0	0	100	0	0	100	0	0	0	0	0	0	0	100	0	0
Actinomyces israelii	12	0	0	92	0	0	0	0	8	0	50	17	33	0	100	0	17	17	17	0	0	50	33	17	0
Actinomyces israelii 1	7	0	0	71	0	0	0	0	0	0	14	86	0	0	71	0	0	0	0	0	14	29	43	0	0
Actinomyces israelii 2	4	0	0	75	0	0	0	0	0	0	25	50	25	0	100	0	25	0	0	0	0	25	75	0	0
Actinomyces israelii fluorescent antibody negative 1 and 2	2	0	0	50	0	0	0	0	0	0	50	0	50	0	100	50	50	0	50	0	0	50	50	0	0
Actinomyces meyeri	39	0	0	3	0	0	0	0	0	0	18	41	41	0	69	10	0	31	15	0	0	0	41	0	0
Actinomyces naeslundii	31	0	0	42	0	0	0	0	0	3	52	26	23	3	97	3	6	0	16	0	6	13	74	10	0
Actinomyces odontolyticus	56	4	0	50	2	2	0	0	0	0	38	36	27	0	95	9	2	5	16	0	0	2	50	25	0
Actinomyces pyogenes	12	0	0	0	0	8	8	0	8	0	0	67	33	0	100	8	92	25	58	8	75	8	25	8	0
Actinomyces viscosus	25	12	0	48	8	84	72	0	0	0	24	28	48	0	92	4	4	4	16	80	8	12	56	4	0
Actinomyces spp.	26	0	0	38	0	15	12	0	0	0	31	35	35	4	65	15	23	8	12	8	4	12	50	4	0
Anaerobiospirillum succiniciproducens	34	0	0	0	0	0	0	0	0	0	62	12	26	0	97	0	3	0	15	0	0	3	59	6	0
Anaerorhabdus furcosus	1	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0
Arcanobacterium haemolyticum	11	0	0	0	0	0	0	0	27	0	9	9	82	0	27	0	0	64	64	0	27	0	36	0	0
Arcobacter nitrofigilis	2	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0	0
Bacteroides caccae	1	0	0	100	0	0	0	0	0	0	100	0	0	0	100	0	0	100	0	0	0	0	100	100	0
Bacteroides capillosus	6	0	0	83	0	0	0	0	0	0	0	17	83	0	17	0	0	33	0	0	0	0	0	0	0
Bacteroides coagulans	1	100	0	0	0	0	0	0	100	0	0	100	0	0	0	0	100	0	100	0	100	0	0	0	0
Bacteroides distasonis	21	0	0	100	0	76	67	0	0	0	86	5	10	5	100	0	24	5	48	38	0	0	100	95	0
Bacteroides forsythus	1	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Bacteroides fragilis	135	0	0	97	0	90	86	1	0	0	100	0	0	24	100	16	15	4	30	14	0	0	100	0	0
Bacteroides gracilis	10	0	0	0	10	0	0	0	0	0	40	0	60	0	0	0	0	0	20	0	0	0	0	0	0
Bacteroides levii	1	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100	100	0	0	100	0	0	0	0
Bacteroides macacae	1	100	0	0	0	100	100	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0	0	0	0
Bacteroides merdae	1	0	0	100	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	100	100	0
Bacteroides microfusus	1	0	0	0	0	100	0	0	0	0	100	0	0	0	0	0	0	0	0	100	0	0	0	0	0
Bacteroides ovatus	5	80	0	100	0	60	40	0	0	0	100	0	0	20	100	40	40	60	20	40	0	80	100	100	0
Bacteroides putredinis	2	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0	50	0	100	100	0	0	0	0
Bacteroides salivosus	1	0	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	100	0	0	100	0	0	0	0
Bacteroides splanchnicus	2	100	0	0	50	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	50	0	100	0	0
Bacteroides stercoris	1	100	0	100	0	0	0	0	0	0	100	0	0	0	100	100	0	100	0	0	0	0	100	100	0
Bacteroides thetaiotaomicron	71	92	0	97	0	86	76	0	0	0	100	0	0	1	100	25	17	79	20	24	3	1	97	96	0
Bacteroides uniformis	5	100	0	80	0	0	0	0	0	0	80	20	0	0	100	0	40	60	0	0	0	0	100	20	0
Bacteroides ureolyticus	1	0	0	0	3	0	0	0	0	0	39	16	45	0	0	3	32	0	13	0	19	0	0	0	0
Bacteroides vulgatus	23	0	0	17	4	0	0	0	0	0	100	0	0	0	100	4	13	9	26	0	0	0	100	100	0
Bacteroides spp.	71	25	0	32	11	18	15	0	0	7	30	8	62	0	61	18	21	35	23	13	17	1	54	13	0
Bifidobacterium adolescentis	8	0	0	38	0	0	0	0	0	0	88	0	13	0	100	25	0	0	13	0	0	25	88	0	0
Bifidobacterium bifidum	4	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	100	0	0
Bifidobacterium breve	4	0	0	75	0	0	0	0	0	0	100	0	0	0	100	25	0	0	50	0	0	50	100	0	0
Bifidobacterium catenulatum	1	0	0	0	0	0	0	0	0	0	0	0	100	0	100	0	0	0	0	0	0	0	100	0	0
Bifidobacterium dentium	1	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	100	100	0	0
Bifidobacterium dentium eriksonii	3	0	0	0	33	0	0	0	0	0	100	0	0	0	100	0	0	0	33	0	0	100	67	0	0
Bifidobacterium infantis	2	0	0	0	0	0	0	0	0	0	100	0	0	0	100	50	0	0	0	0	0	0	100	0	0
Bifidobacterium longum	1	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	100	0	0
Bifidobacterium spp.	16	0	0	38	0	0	0	0	0	0	88	6	6	0	75	19	0	0	0	0	0	38	69	6	0
Clostridium absonum	4	0	0	100	0	0	0	100	0	0	100	0	0	0	100	0	0	0	100	0	100	0	100	0	0
Clostridium baratii	8	0	0	100	0	0	0	100	0	0	88	0	13	0	100	38	38	0	63	0	0	0	75	0	0
Clostridium beijerinckii	14	0	0	100	0	0	0	0	0	0	7	0	93	0	100	50	36	0	64	0	0	79	79	0	0
Clostridium bifermentans	16	100	0	88	6	0	0	100	0	0	94	0	6	0	88	0	81	6	31	0	100	0	0	0	0

Continued on following page

TABLE 3—*Continued*

Species	No. of strains	% Positive reactions on differential media from: CDC Presumpto Plate I: Indole	Indole derivative	Esculin	H_2S-esculin	Catalase-LD	Catalase-esculin	Lecithinase	Lipase	Egg yolk agar proteolysis	Bile agar: E growth	Bile agar: I growth	Bile agar: No growth	Bile agar: Precipitate	CDC Presumpto Plate II: Glucose	Starch hydrolysis	Proteolysis of milk	DNase	DNA clearing	Catalase-milk	CDC Presumpto Plate III: Gelatin	Mannitol	Lactose	Rhamnose	Pink with HgCl
Clostridium botulinum A	1	0	100	100	0	0	0	0	100	0	0	100	0	0	100	0	100	0	0	0	100	0	0	0	0
Clostridium botulinum B	3	0	100	67	0	0	0	0	100	0	100	0	0	0	100	0	67	67	0	0	67	0	0	0	0
Clostridium botulinum E	2	0	50	0	0	0	0	0	100	0	50	0	50	0	100	0	0	50	0	0	50	0	0	0	0
Clostridium botulinum G	1	0	100	0	100	0	0	0	0	0	0	100	0	0	0	0	100	0	0	0	100	0	0	0	0
Clostridium butyricum	31	0	0	100	0	0	0	0	0	0	81	16	3	0	100	74	0	0	58	0	0	10	100	0	0
Clostridium cadaveris	6	83	17	0	67	0	17	0	0	0	83	0	17	0	100	0	0	100	0	0	33	0	0	0	17
Clostridium carnis	9	0	0	89	0	0	0	0	0	0	89	0	11	0	100	0	0	0	89	0	0	0	44	0	0
Clostridium celatum	2	0	0	100	0	0	0	0	0	0	50	0	50	0	100	50	0	0	0	0	0	0	50	0	0
Clostridium chauvoei	5	0	0	0	0	0	0	0	0	0	100	0	0	0	20	0	80	0	40	0	80	0	0	0	0
Clostridium clostridioforme	18	22	0	67	0	0	0	0	0	0	50	22	28	0	83	11	11	22	6	0	6	0	72	50	0
Clostridium difficile	490	0	0	97	0	0	0	0	0	0	100	0	0	0	100	0	1	0	2	0	0	99	0	0	0
Clostridium fallax	1	0	0	100	0	0	0	0	0	0	100	0	0	0	100	0	0	100	100	0	0	0	0	0	0
Clostridium haemolyticum	1	0	0	0	0	0	0	100	0	0	0	0	100	0	100	0	0	0	0	0	0	0	0	0	0
Clostridium histolyticum	5	0	0	0	0	0	0	0	0	0	60	20	20	0	0	0	100	0	100	0	80	0	0	0	0
Clostridium innocuum	18	0	0	89	0	0	0	0	0	0	100	0	0	0	100	0	0	0	50	0	0	83	11	0	0
Clostridium limosum	2	0	0	0	0	0	0	0	0	0	0	0	100	0	50	50	50	50	0	0	0	0	0	0	0
Clostridium lituseburense	1	0	0	0	0	0	0	100	0	0	100	0	0	0	100	0	100	0	0	0	100	0	0	0	0
Clostridium malenominatum	7	100	0	0	43	0	0	0	0	0	29	29	43	0	14	0	0	29	14	0	0	0	0	0	0
Clostridium novyi	1	0	0	0	0	0	0	100	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Clostridium novyi A	7	0	0	0	0	0	0	86	100	14	14	0	86	0	57	0	14	0	0	0	57	0	0	0	0
Clostridium novyi B	1	0	0	0	0	0	0	100	0	0	0	0	100	0	100	0	0	0	0	0	0	0	0	0	0
Clostridium paraputrificum	13	0	0	92	0	0	0	0	0	0	100	0	0	0	100	23	0	0	54	0	0	8	92	0	0
Clostridium perfringens	79	0	1	30	22	0	0	94	0	0	100	0	0	0	99	73	53	92	23	1	91	0	100	0	0
Clostridium putrificum	2	0	0	0	0	0	0	0	0	0	100	0	0	0	50	0	100	0	100	0	100	0	0	0	0
Clostridium ramosum	16	0	0	94	0	0	0	0	0	0	100	0	0	0	100	0	13	0	19	0	0	38	94	31	0
Clostridium septicum	56	0	0	79	0	2	2	0	0	0	95	2	4	0	95	0	7	88	7	0	79	0	82	0	0
Clostridium sordellii	32	91	3	6	38	0	0	84	0	0	100	0	0	0	97	0	91	9	19	3	97	0	0	0	0
Clostridium sphenoides	8	75	0	50	0	0	0	0	0	0	38	25	38	0	100	0	0	50	13	0	0	63	38	50	13
Clostridium spiroforme	2	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	100	0	0	0	0	0	50	0	0
Clostridium sporogenes	52	2	90	94	2	0	0	17	90	2	92	8	0	0	94	0	96	25	12	0	96	0	0	0	0
Clostridium subterminale	7	0	0	0	43	0	0	0	0	0	57	29	14	0	0	14	57	14	14	0	71	0	0	0	0
Clostridium symbiosum	2	0	0	0	0	0	0	0	0	0	0	50	50	0	50	0	0	0	50	0	0	50	0	0	0
Clostridium tertium	44	0	0	98	0	0	0	0	0	0	98	0	2	0	100	52	2	84	7	0	0	93	100	2	0
Clostridium tetani	27	70	0	4	56	0	0	0	15	0	70	26	4	0	0	4	0	59	4	0	93	0	0	0	0
Clostridium spp.	121	15	2	45	8	2	3	18	6	0	64	9	26	0	70	8	15	16	21	2	31	15	29	5	0
Corynebacterium group e	1	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Corynebacterium matruchotii	7	0	0	43	0	71	71	0	0	0	29	29	43	0	86	29	0	0	0	86	0	14	0	0	0
Corynebacterium spp.	4	0	0	25	0	100	100	0	0	0	75	0	25	50	25	0	0	0	0	100	0	25	25	25	0
Desulfomonas pigra	12	0	0	8	25	58	42	0	0	0	42	0	58	0	0	0	0	0	0	58	0	0	0	0	0
Desulfovibrio desulfuricans	20	0	0	0	25	70	55	0	0	0	45	30	25	0	0	0	0	0	5	65	0	0	0	0	0
Dichelobacter nodosus	1	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	0
Eikenella corrodens	2	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Eubacterium aerofaciens	3	0	0	33	0	0	0	0	0	0	100	0	0	0	100	0	67	0	0	0	0	0	100	0	0
Eubacterium alactolyticum	4	0	0	0	0	0	0	0	0	0	0	75	25	0	50	0	0	0	0	0	0	0	0	0	0
Eubacterium brachy	2	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Eubacterium budayi	1	0	0	100	0	0	0	100	0	0	100	0	0	0	100	100	0	100	0	0	0	0	100	0	0
Eubacterium combesii	1	0	100	100	0	0	0	0	100	0	100	0	0	0	100	0	0	100	0	0	100	0	0	0	0
Eubacterium cylindroides	1	0	0	0	0	0	0	0	0	0	100	0	0	0	100	100	0	0	0	0	100	0	0	0	0
Eubacterium dolichum	1	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Eubacterium formicigenerans	1	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Eubacterium lentum	33	0	0	0	24	58	36	0	0	0	67	9	24	0	0	0	0	0	3	45	3	0	0	0	0
Eubacterium limosum	2	0	0	0	100	0	0	0	0	0	100	0	0	0	100	50	0	0	0	0	0	100	0	0	0
Eubacterium nodatum	3	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Eubacterium saburreum	2	50	0	50	0	0	0	0	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	0	0
Eubacterium timidum	3	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Eubacterium spp.	2	0	0	0	0	50	50	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	50	0	0

Continued on following page

TABLE 3—*Continued*

Species	No. of strains	% Positive reactions on differential media from: CDC Presumpto Plate I: Indole	Indole derivative	Esculin	H_2S-esculin	Catalase-LD	Catalase-esculin	Lecithinase	Lipase	Egg yolk agar proteolysis	Bile agar: E growth	Bile agar: I growth	Bile agar: No growth	Bile agar: Precipitate	CDC Presumpto Plate II: Glucose	Starch hydrolysis	Protcolysis of milk	DNase	DNA clearing	Catalase-milk	CDC Presumpto Plate III: Gelatin	Mannitol	Lactose	Rhamnose	Pink with HgCl
Fusobacterium gonidiaformans	6	83	0	0	17	0	0	0	0	0	0	17	83	0	33	0	0	0	0	0	0	0	0	0	0
Fusobacterium mortiferum	5	20	0	100	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	60	0	0
Fusobacterium naviforme	6	100	0	0	0	0	0	0	0	0	17	0	83	0	17	0	0	0	17	0	0	0	0	0	0
Fusobacterium necrogenes	1	0	0	0	100	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0
Fusobacterium necrophorum	18	100	0	0	56	0	0	0	100	0	11	11	78	0	67	0	89	17	22	0	17	0	0	0	6
Fusobacterium nucleatum	31	97	3	0	39	0	0	0	3	0	3	10	87	0	39	0	3	0	10	0	0	0	0	0	0
Fusobacterium periodonticum	1	100	0	0	0	0	0	0	0	0	0	0	100	0	100	0	0	0	0	0	0	0	0	0	0
Fusobacterium russii	3	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Fusobacterium simiae	1	100	0	0	100	0	0	0	100	100	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0
Fusobacterium sulci	1	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Fusobacterium varium	6	67	0	0	33	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0
Fusobacterium spp.	11	73	0	27	45	0	0	0	9	0	36	27	36	0	91	18	9	9	18	0	9	9	27	0	0
Mitsuokella multiacidus	1	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	100	100	100	0
Mobiluncus curtisii subsp. *curtisii*	20	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	10	0	0	0	0	0	0
Mobiluncus curtisii subsp. *holmesii*	32	0	0	0	0	0	0	0	0	0	0	16	84	0	0	0	0	0	3	3	0	0	0	0	0
Mobiluncus mulieris	18	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	6	0	0	0	0	0	0
Mobiluncus spp.	9	0	0	0	0	0	0	0	11	0	11	0	89	0	0	0	0	0	0	0	0	0	0	0	0
Peptostreptococcus anaerobius	21	5	0	0	43	0	0	0	0	0	24	38	38	0	67	5	0	0	10	0	0	5	0	5	0
Peptostreptococcus asaccharolyticus	22	91	0	5	0	5	0	0	0	0	18	55	27	0	0	0	0	0	18	5	0	0	0	0	0
Peptostreptococcus indolicus	6	100	0	0	33	0	0	0	0	0	17	50	33	0	0	0	0	0	17	0	0	0	0	0	0
Peptostreptococcus magnus	21	0	0	0	0	29	19	0	5	0	5	76	19	0	0	0	5	0	5	29	0	0	0	0	0
Peptostreptococcus magnus or *P. micros*	4	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Peptostreptococcus micros	1	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Peptostreptococcus prevotii	15	0	0	0	0	13	13	0	0	0	33	33	33	0	0	0	0	0	7	0	0	0	0	0	0
Peptostreptococcus productus	2	0	0	50	0	0	0	0	0	0	100	0	0	0	50	0	0	0	0	0	0	50	50	50	0
Peptostreptococcus tetradius	1	0	0	0	0	100	100	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Peptostreptococcus spp.	2	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Porphyromonas asaccharolytica	6	67	0	0	0	0	0	0	17	0	0	0	100	0	0	0	83	17	0	0	67	0	0	0	0
Porphyromonas endodontalis	1	100	0	0	0	0	0	0	0	0	0	0	100	0	100	0	0	0	0	0	100	0	0	0	0
Porphyromonas gingivalis	13	92	8	0	31	0	0	0	0	54	0	0	100	0	0	15	100	0	62	0	100	0	0	0	0
Prevotella bivia	14	0	0	0	0	0	0	0	0	0	0	7	93	0	93	14	71	86	0	7	57	14	100	21	0
Prevotella corporis	1	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	0	0	0	0
Prevotella denticola	5	0	0	20	0	0	0	0	0	0	0	0	100	0	100	100	0	80	0	0	20	0	100	0	0
Prevotella disiens	3	0	0	0	0	0	0	0	0	0	33	0	67	0	67	0	100	67	0	0	100	0	0	0	0
Prevotella heparinolytica	1	0	0	100	0	0	0	0	0	0	0	0	100	0	100	0	0	0	0	0	0	0	100	0	0
Prevotella intermedia	16	88	0	0	6	0	0	0	94	31	6	6	88	0	69	63	88	100	13	0	94	0	6	0	0
Prevotella loescheii	6	0	0	33	0	0	0	0	67	33	0	0	100	0	67	67	50	50	67	0	50	0	67	0	0
Prevotella melaninogenica	10	0	0	0	10	0	0	0	0	0	0	10	90	0	100	100	0	80	30	0	60	0	100	0	0
Prevotella oralis	2	0	0	100	0	0	0	0	0	0	0	0	100	0	50	50	0	50	50	0	0	0	50	50	0
Prevotella oris or *P. buccae*	9	0	0	89	0	0	0	0	0	0	0	11	89	0	100	100	78	89	22	0	0	0	100	22	0
Prevotella oulora	1	0	0	0	0	100	100	0	0	0	100	0	0	100	0	0	0	0	100	100	0	0	0	0	0
Prevotella veroralis	2	0	0	50	0	0	0	0	0	0	0	0	100	0	100	100	0	50	0	0	0	0	100	50	0
Prevotella zoogleoformans	5	0	0	80	0	0	0	0	0	0	0	0	100	0	80	80	0	0	0	0	0	0	60	0	0
Propionibacterium acidipropionici	1	0	0	100	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	100	100	100	0
Propionibacterium acnes	144	80	0	3	0	94	44	0	22	0	62	19	19	0	87	2	75	3	9	88	67	4	3	0	14
Propionibacterium avidum	5	0	0	80	0	100	80	0	80	0	100	0	0	0	100	0	60	0	20	100	80	0	0	0	0
Propionibacterium freudenreichii	1	0	0	0	0	100	100	0	0	0	100	0	0	0	100	0	0	0	0	100	0	0	0	0	0

Continued on following page

TABLE 3—*Continued*

Species	No. of strains	% Positive reactions on differential media from: CDC Presumpto Plate I: Indole	Indole derivative	Esculin	H_2S-esculin	Catalase-LD	Catalase-esculin	Lecithinase	Lipase	Egg yolk agar proteolysis	Bile agar: E growth	Bile agar: I growth	Bile agar: No growth	Bile agar: Precipitate	CDC Presumpto Plate II: Glucose	Starch hydrolysis	Proteolysis of milk	DNase	DNA clearing	Catalase-milk	CDC Presumpto Plate III: Gelatin	Mannitol	Lactose	Rhamnose	Pink with HgCl
Propionibacterium granulosum	7	0	0	0	0	86	43	0	29	0	57	29	14	0	100	0	57	29	0	100	29	29	14	0	14
Propionibacterium jensenii	1	0	0	100	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	100	100	0	0
Propionibacterium lymphophilum	1	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0
Propionibacterium propionicus	10	0	0	0	0	0	0	0	0	0	10	40	50	0	60	0	0	0	40	0	10	50	50	0	0
Propionibacterium thoenii	1	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0
Propionibacterium spp.	11	0	0	9	0	45	9	0	18	0	36	27	36	0	36	0	0	0	0	36	18	0	9	0	0
Wolinella curva H_2S negative on triple sugar iron	1	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wolinella curva or *W. succinogenes*	1	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Wolinella succinogenes	5	0	0	0	20	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Wolinella or *Campylobacter* spp.	3	0	0	0	0	0	0	0	0	0	67	0	33	0	0	0	0	0	0	0	0	0	0	0	0
Wolinella spp.	20	0	0	0	10	0	0	0	0	0	25	10	65	0	0	0	0	0	15	5	0	0	0	0	0

it takes less than 2 min to inoculate and prepare the three plates for incubation.

Clearly, some strains listed in the identification chart (Table 3) are rare, and we were unable to test more of these isolates. Therefore, the Presumpto Plate reactions of these strains should be interpreted with caution. An octal code system is being developed for use with the chart to assist in the rapid recognition of organisms based on their reactions in Presumpto Plates.

The information derived from colony characteristics, Gram stain, morphologic features, sporulation, flagellation, hemolysis, fluorescence with long-wave UV light, acid products identified by gas-liquid chromatography, and Presumpto Plates I, II, and III allows definitive identification of most anaerobic bacteria of clinical significance likely to be encountered in a clinical laboratory. Because conventional media were used, there was a high degree of agreement between the Presumpto Plate method and the reference method when testing commonly encountered anaerobes. The Presumpto Plate is as accurate as commercially available enzyme systems for the identification of many anaerobic species but is less expensive to perform (unpublished data).

TABLE 4. Separation of *Bacteroides* species by using Presumpto Plates

Bacteroides species	Result[a] with: Indole	Mannitol	Rhamnose
B. distasonis	−	−	V
B. fragilis	−	−	−
B. ovatus	+	A	A
B. thetaiotaomicron	+	−	A
B. vulgatus	−	−	A

[a] −, negative; +, positive; V, variable; A, acid.

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Related Articles, Links

Aerobic and anaerobic microbiology of dacryocystitis.

Brook I, Frazier EH.

Department of Pediatrics, Navy Hospital, USA.

PURPOSE: To investigate the aerobic and anaerobic microbiology of dacryocystitis. METHOD: Retrospective review of the 62 clinical and microbiologic records collected between 1980 and 1990. RESULTS: Aerobic or facultative bacteria were recovered in 32 cases (52%), anaerobic bacteria only in 20 cases (32%), mixed aerobic and anaerobic bacteria in seven cases (11%), and fungi in three cases (5%). A total of 94 organisms (1.5 per specimen), which included 56 aerobic or facultative anaerobic organisms, 35 anaerobic organisms, and three fungi, were recovered. The predominant aerobic and facultative bacteria were Staphylococcus aureus (15 isolates), Staphylococcus epidermidis (13 isolates), and Pseudomonas species (seven isolates). The most frequently recovered anaerobes were Peptostreptococcus species (13 isolates), Propionibacterium species (12 isolates), Prevotella species (four isolates), and Fusobacterium species (three isolates). The predominant fungus was Candida albicans (two isolates). Polymicrobial infection was present in 28 cases (45%). CONCLUSION: These data highlight the potential importance of anaerobic bacteria in dacryocystitis.

PMID: 9559743 [PubMed - indexed for MEDLINE]

Antibiot Med Biotekhnol. 1987 Oct;32(10):764-7.

Related Articles, Links

[Method for demonstrating strict anaerobes in microbial associations]

[Article in Russian]

Bazhenov LG, Iskhakova KhI.

A procedure for indicating strict anaerobes in microbial associations was developed. The indication is performed with two disks: aminoglycoside (amikacin, 10-micrograms disk) (A) and metronidazole aminoglycoside (metronidazole + amikacin, 5- and 10-micrograms disks respectively) (MA). Colonies growing in zone A and absent in zone MA are considered to be strictly anaerobic. The procedure is characterized by high reliability: false positive results are practically not recorded and the frequency of false negative results does not exceed 5-10 per cent. The rate of detecting anaerobes in clinical materials is significantly increased (by 1-3 days) as compared to that with the use of the routine bacterial methods. The indication procedure markedly simplifies laboratory diagnosis of anaerobic infections at the account of clear differentiation of microorganisms grown on the primary plate and possible directed selection of anaerobic colonies for further investigation which excludes the necessity of rejecting or studying other colony types. This leads to economy of nutrient media and increasing labor productivity.

PMID: 3322186 [PubMed - indexed for MEDLINE]

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STABILIZATION OF ISOLATED PHOTOSYSTEM II REACTION CENTER COMPLEX IN THE DARK AND IN THE LIGHT USING POLYETHYLENE GLYCOL AND AN %OXYGEN%-%SCRUBBING% SYSTEM
AUTHOR: MCTAVISH H (Reprint); PICOREL R; SEIBERT M
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JOURNAL: %Plant Physiology (Rockville)% 89 (2): p452-456 %1989%
ISSN: 0032-0889
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LANGUAGE: ENGLISH

ABSTRACT: The photosystem II reaction center [of spinach membrane fragments] as isolated (O Nanba, K Satoh [1987] Proc Natl Acad Sci USA 84: 109-112) is quite dilute and very unstable. Precipitating the complex with polyethylene glycol and resuspending it in buffer without detergent concentrates the reaction center and greatly improves its stability at 4° C in the dark as judged by light-induced electron transport activity. Furthermore, a procedure was developed to minimize photodestruction of polyethylene-glycol-concentrated material at room temperature in the light. The ability to stabilize the photosystem II reaction center should facilitate future photophysical biochemical, and structural studies of the complex.

REGISTRY NUMBERS: 25322-68-3: POLYETHYLENE GLYCOL
DESCRIPTORS: SPINACH MEMBRANE FRAGMENTS ELECTRON TRANSPORT PHOTODESTRUCTION
DESCRIPTORS:
MAJOR CONCEPTS: Bioenergetics--Biochemistry and Molecular Biophysics; Cell Biology; Metabolism; Radiation Biology
BIOSYSTEMATIC NAMES: Chenopodiaceae--Dicotyledones, Angiospermae, Spermatophyta, Plantae
COMMON TAXONOMIC TERMS: Angiosperms; Dicots; Plants; Spermatophytes; Vascular Plants
CHEMICALS & BIOCHEMICALS: POLYETHYLENE GLYCOL
CONCEPT CODES:
02504 Cytology - Plant
10012 Biochemistry - Gases
10064 Biochemistry studies - Proteins, peptides and amino acids
10065 Biochemistry studies - Porphyrins and bile pigments
10068 Biochemistry studies - Carbohydrates
10508 Biophysics - Membrane phenomena
10510 Biophysics - Bioenergetics: electron transport and oxidative phosphorylation
10604 External effects - Light and darkness
10614 External effects - Temperature as a primary variable
13003 Metabolism - Energy and respiratory metabolism
51506 Plant physiology - Photosynthesis
51516 Plant physiology - Light and radiation effects
BIOSYSTEMATIC CODES:

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Use of the BacT/Alert Blood Culture System for Culture of Sterile Body Fluids Other than Blood

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Studies have demonstrated that large-volume culture methods for sterile body fluids other than blood increase recovery compared to traditional plated-medium methods. BacT/Alert is a fully automated blood culture system for detecting bacteremia and fungemia. In this study, we compared culture in BacT/Alert standard aerobic and anaerobic bottles, BacT/Alert FAN aerobic and FAN anaerobic bottles, and culture on routine media for six specimen types, i.e., continuous ambulatory peritoneal dialysate (CAPD), peritoneal, amniotic, pericardial, synovial, and pleural fluids. Specimen volumes were divided equally among the three arms of the study. A total of 1,157 specimens were tested, with 227 significant isolates recovered from 193 specimens. Recovery by method was as follows: standard bottles, 186 of 227 (82%); FAN bottles, 217 of 227 (96%); and routine culture, 184 of 227 (81%). The FAN bottles recovered significantly more gram-positive cocci ($P < 0.001$), *Staphylococcus aureus* ($P = 0.003$), coagulase-negative staphylococci ($P = 0.008$), gram-negative bacilli ($P < 0.001$), *Enterobacteriaceae* ($P = 0.005$), and total organisms ($P < 0.001$) than the routine culture. There were no significant differences in recovery between the standard bottles and the routine culture. The FAN aerobic bottle recovered significantly more gram-positive cocci ($P < 0.001$), *S. aureus* isolates ($P < 0.001$), coagulase-negative staphylococci ($P = 0.003$), and total organisms ($P < 0.001$) than the standard aerobic bottle, while the FAN anaerobic bottle recovered significantly more gram-positive cocci ($P < 0.001$), *S. aureus* isolates ($P < 0.001$), *Enterobacteriaceae* ($P = 0.03$), and total organisms ($P < 0.001$) than the standard anaerobic bottle. For specific specimen types, significantly more isolates were recovered from the FAN bottles compared to the routine culture for synovial ($P < 0.001$) and CAPD ($P = 0.004$) fluids. Overall, the FAN bottles were superior in performance to both the standard bottles and the routine culture for detection of microorganisms from the types of sterile body fluids included in this study.

The traditional method for culture of sterile body fluids other than blood involves culture on solid medium with or without an enrichment broth, such as thioglycolate broth. Concentration of specimens is accomplished by filtration or centrifugation.

For some types of body fluids, other large-volume culture methods have been evaluated, including culture in blood culture bottles. Continuous ambulatory peritoneal dialysate (CAPD) specimens are particularly well-suited to large-volume culture techniques, because specimen volume is often very large, while the concentration of organisms can be relatively low. Several commercial blood culture systems, including Bactec (Becton Dickinson Microbiology Systems, Cockeysville, Md.), Septi-Chek (Becton Dickinson Microbiology Systems), and Isolator (Wampole Laboratories, Cranbury, N.J.), have been used for CAPD culture (4, 5, 13, 17). The use of blood culture bottles has also been shown to be superior to conventional culture for the diagnosis of spontaneous bacterial peritonitis (3). More-limited studies have also suggested a role for culturing of synovial fluids in blood culture bottles, particularly for pediatric patients (12, 18).

The BacT/Alert system is a continuously monitored blood culture system for detecting bacteremia and fungemia (10). In addition to the standard BacT/Alert aerobic and anaerobic blood culture bottles, new media, designated FAN aerobic and FAN anaerobic bottles, are available. FAN bottles have been shown to enhance the recovery of fastidious bacteria, bacteria from patients receiving antimicrobial therapy, and yeasts in comparison to the standard BacT/Alert bottles (15, 16).

Although the BacT/Alert system has been thoroughly evaluated for culturing of blood, only a limited number of studies have evaluated the utility of this method for culturing of other types of sterile body fluids (1, 2, 11). The present study was designed to assess the performance of the BacT/Alert system to recover microorganisms from several types of sterile body fluids with standard aerobic and anaerobic bottles and FAN aerobic and FAN anaerobic bottles versus conventional media. Additionally, we wanted to determine whether there was any difference in recovery between the BacT/Alert FAN bottles and the standard BacT/Alert bottles.

MATERIALS AND METHODS

All specimens were collected from patients at Geisinger Medical Center, Danville, Pa.; The Reading Hospital and Medical Center, Reading, Pa.; or the University of Michigan Medical Center, Ann Arbor, Mich.

Specimen types included in this study were pleural, peritoneal, pericardial, amniotic, and synovial fluids and CAPD. Only specimens with a minimum volume of 3.0 ml were included. A maximum volume of 60 ml was utilized, even when more specimen was available. All specimens were collected by standard protocols for collection of sterile fluids at the three participating institutions.

The specimens were divided into three equal aliquots. One aliquot was divided equally between one set of standard BacT/Alert aerobic and anaerobic bottles, the second aliquot was divided equally between one set of BacT/Alert FAN aerobic and FAN anaerobic bottles, and the third aliquot was used to inoculate the routine bacteriology media. When the volume for routine culture was equal

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TABLE 1. Comparative yield of clinically significant isolates of bacteria and yeast by specimen type

Specimen type (total specimens)	No. of positive specimens (% positive)	No. of total isolates	No. of isolates (% of total no. of isolates) recovered from:			*P* for FAN versus routine method
			Routine culture	Standard bottles	FAN bottles	
Peritoneal (209)	46 (22.0)	66	52	51	59	NS[a]
Pleural (241)	10 (4.1)	13	11	9	13	NS
Synovial (361)	50 (13.8)	51	37	40	51	<0.001
CAPD (287)	85 (29.6)	95	82	84	92	0.004
Amniotic (15)	0 (0.0)	0	0	0	0	NC[b]
Pericardial (27)	2 (7.8)	2	2	2	2	NC
Total (1,140)	193 (16.9)	227	184 (81.0)	186 (86.3)	217 (95.6)	<0.001

[a] NS, not significant ($P > 0.05$).
[b] NC, not calculated when total number of isolates was ≤10.

to 1 ml, the specimen was divided among the plated media, thioglycolate broth, and a slide for Gram staining. Specimen types were placed into three groups for plating on routine medium. Synovial and pericardial fluids (group 1) were plated on blood agar plates, chocolate agar plates, and thioglycolate broth with vitamin K and hemin. CAPDs (group 2) were plated on the group 1 media plus MacConkey agar plates. Peritoneal, pleural, and amniotic fluids (group 3) were plated on the group 2 media plus Columbia nalidixic acid agar plates and anaerobic blood agar plates, phenylethyl alcohol agar plates, and laked kanamycin-vancomycin agar plates. When the volume for routine culture was >1.0 ml, the specimen was centrifuged, resuspended in 1.0 ml of supernatant, and plated in the manner used for the 1.0-ml specimens.

After venting of the standard aerobic and FAN aerobic bottles, all bottles were loaded into BacT/Alert instruments. The instruments were the same instruments utilized in the laboratories for routine blood cultures. The standard BacT/Alert software was used. Bottles flagged as positive by the BacT/Alert system were subcultured and interpreted according to the standard protocols for each of the participating laboratories. For the purposes of this study, each bottle was processed independently of the other three bottles in a set, i.e., a negative bottle was not examined when another bottle in a set was flagged as positive (except as noted below for terminal subcultures). All BacT/Alert bottles were incubated for 7 days at The Reading Hospital and Medical Center and the University of Michigan Medical Center and for 6 days at Geisinger Medical Center.

When growth was detected on routine media or in one or more of the bottles from one specimen but not in the other bottle(s) inoculated from the same specimen, terminal subcultures were performed on the negative bottles at the end of the standard incubation period. Aerobic and anaerobic bottles were subcultured on chocolate agar plates incubated aerobically in a 5 to 10% CO_2-enriched atmosphere. Anaerobic bottles were also subcultured on blood agar plates incubated anaerobically. Approximately 20% of all other negative sets of bottles were also blindly subcultured on the same media to establish an accurate false-negative rate.

Routine plated and tubed media were incubated at 35°C in either 5 to 10% CO_2 (aerobic culture) or anaerobically (anaerobic culture). The routine media were examined by the standard protocols in use at each of the participating laboratories. Aerobic cultures were incubated for a minimum of 2 days, while anaerobic cultures were maintained for a minimum of 5 days. Bacterial identification and antimicrobial susceptibility testing were performed according to standard laboratory protocols.

Chart review was conducted by the principal investigator at each site to determine which isolates were clinically significant.

Statistical analyses were carried out by methods described by Ilstrup (6).

RESULTS

A total of 1,157 specimens were included in this study (Table 1). In one of our laboratories, we limit the workup of specimens containing more than three different organisms. Indeed, that was the reason why specimens with more than three organisms were excluded from this study. Seventeen specimens (14 peritoneal, 1 amniotic, 1 pleural, and 1 CAPD) each grew four or more different organisms on the routine culture and were excluded from further analysis. Of the remaining 1,140 specimens, 284 were positive by one or more methods, including 185 which grew clinically significant microorganisms, 91 which grew one or more microorganisms which were not clinically significant, 7 which grew a significant isolate(s) mixed with a microorganism which was not clinically significant, and 1 which grew a significant isolate mixed with an isolate the significance of which could not be determined. In all, there were 227 significant isolates.

Of the 227 significant isolates, 184 (81.0%) grew in the routine culture, 186 (81.9%) grew in the standard bottles, and 217 (95.6%) grew in the FAN bottles. No significant differences were noted between the yields of the routine culture and the standard bottle culture for either individual specimen types or cumulatively. However, significantly more isolates were recovered from the FAN bottles than from the routine cultures for synovial ($P < 0.001$) and CAPD ($P = 0.004$) specimens as well as for total specimens ($P < 0.001$). A trend toward significance was noted for peritoneal fluids favoring the FAN bottles over the routine culture ($P = 0.09$).

A summary of significant isolates is presented in Table 2. No significant differences in organism detection were noted between the routine culture and standard bottle culture for specific microorganisms. However, in comparing the recovery of specific microorganisms between the routine culture and the FAN bottle culture, significantly more gram-positive cocci ($P < 0.001$), *S. aureus* isolates ($P = 0.003$), non-*S. aureus Staphylococcus* sp. isolates ($P = 0.008$), gram-negative bacilli ($P < 0.001$), *Enterobacteriaceae* ($P = 0.005$), and total organisms ($P < 0.001$) were recovered from the FAN bottles than from the routine culture.

A summary of the significant isolates from each of the four bottle types is presented in Table 3. The FAN aerobic bottle recovered significantly more yeast than the FAN anaerobic bottle ($P < 0.001$), while the FAN anaerobic bottle recovered significantly more anaerobic bacteria than the FAN aerobic bottle ($P = 0.003$). The standard aerobic bottle recovered significantly more yeast than the standard anaerobic bottle ($P < 0.001$).

In comparing each FAN bottle to its standard counterpart (Table 3), significant differences were noted. The FAN aerobic bottle recovered significantly more gram-positive cocci ($P < 0.001$), *S. aureus* isolates ($P < 0.001$), non-*S. aureus Staphylococcus* sp. isolates ($P = 0.003$), and total isolates ($P < 0.001$) than the standard aerobic bottle. The FAN anaerobic bottle recovered significantly more gram-positive cocci ($P < 0.001$), *S. aureus* isolates ($P < 0.001$), *Enterobacteriaceae* ($P = 0.03$), and total isolates ($P < 0.001$) than the standard anaerobic bottle.

Blind subcultures were performed on all bottles read as negative by the instrument for which another bottle(s) or the routine culture was positive. For the aerobic bottles, terminal subculture detected one *Candida albicans* isolate from a FAN bottle and one *C. albicans* isolate and one *S. aureus* isolate from standard aerobic bottles. Terminal subculture of the standard anaerobic bottles detected three *C. albicans*, one *Candida tropicalis*, one coagulase-negative staphylococcus, and two *Fla-*

vobacterium odoratum isolates, while terminal subculture of the anaerobic FAN bottle detected five *C. albicans* isolates, one *Candida parapsilosis* isolate, one *Candida tropicalis* isolate, one *Pseudomonas aeruginosa* isolate, two *Flavobacterium odoratum* isolates, and one coagulase-negative staphylococcus isolate.

In addition, blind terminal subcultures were performed on all four bottles from 192 specimens, with negative results by all methods. No additional isolates were detected by these subcultures.

Three bottles were flagged as positive by the BacT/Alert instruments, but no organisms were seen by Gram staining and no organisms grew from subculture of the bottles. These three bottles were classified as false-positive results. The false-positive test results did not appear to be related to the specimen volume in these three bottles. Two of the bottles had ≤2.5 ml, while the third bottle had 5.01 to 7.5 ml. There were no false-positive results from the 157 specimens (628 bottles) containing the largest inoculum volumes, i.e., 7.51 to 10 ml.

DISCUSSION

This study was undertaken to compare the BacT/Alert system with a routine culture method for the recovery of microorganisms from six types of sterile body fluids other than blood. Although it has been marketed and cleared by the Food and Drug Administration for the detection of microorganisms from blood, the reported success of other blood culture methods and systems in the culture of body fluids other than blood prompted us to evaluate the BacT/Alert system for this purpose.

The results of this study show that the standard BacT/Alert bottles are equivalent to a rigorous, routine culture method for the recovery of bacteria and yeast from sterile body fluids other than blood.

The yield of the FAN bottles in this study was superior to those of both the standard bottles and the routine culture. The increased yield of the FAN bottles in comparison to that of the standard bottles in this study with body fluids other than blood was similar to published results obtained with blood (15, 16).

When blood is cultured in blood culture bottles, the blood itself provides some of the nutrients required for the growth of fastidious microorganisms, such as *Neisseria gonorrhoeae*. It is doubtful that an unsupplemented blood culture bottle used for the culture of body fluids other than blood can support the growth of all fastidious microorganisms. Fuller et al. evaluated the recovery of microorganisms from sterile body fluids in a study which compared a routine culture method with the Bactec Plus 26/27 culture system (5). The Bactec bottles were evaluated with and without a fastidious supplement. They recovered one isolate of *N. gonorrhoeae* and two isolates of *Haemophilus influenzae* only from the supplemented bottles. In this study, we recovered no isolates of *N. gonorrhoeae*. One isolate of *H. influenzae* was recovered from a FAN anaerobic bottle only.

An alternative to supplementing the bottles could be the use of a single chocolate agar plate. Indeed, the frequency with which such fastidious organisms might be isolated from specific types of fluids may influence the decision as to whether an unsupplemented bottle could serve as a stand-alone culture medium. For example, *N. gonorrhoeae* is a rare cause of CAPD infections but occurs more frequently in synovial fluids; thus, a chocolate agar plate might be added to a blood culture bottle for a synovial fluid but not for a CAPD fluid. Additional studies are needed to permit a more-accurate assessment of the need for either a fastidious supplement to the blood culture media or supplementary solid media for various specimen types.

TABLE 2. Comparative yield of clinically significant isolates of bacteria and yeast

Microorganism	Total no. of isolates	No. of isolates from:			*P* for FAN vs routine method
		Routine culture	Standard bottles	FAN bottles	
Gram-positive cocci	138	111	111	133	<0.001
S. aureus	51	42	39	51	0.003
Coagulase-negative staphylococcus	50	41	42	48	0.008
Enterococci	18	13	14	16	NS[e]
Streptococci[a]	19	15	16	18	NS
Gram-positive bacilli	4	3	4	3	NC[f]
Corynebacterium sp.	3	2	3	2	NC
Listeria sp.	1	1	1	1	NC
Gram-negative bacilli	54	42	46	53	<0.001
Enterobacteriaceae[b]	39	31	33	39	0.005
Other GNB[c]	15	11	13	14	NS
Anaerobes	9	7	5	9	NC
Clostridium sp.	5	3	2	5	NC
Anaerobic GNB	4	4	3	4	NC
Fungi					
Yeast[d]	22	21	20	19	NS
All microorganisms	227	184	186	217	<0.001

[a] Includes four *S. pneumoniae* isolates; one group B, three group G, and seven *viridans* group streptococci; two *S. milleri* group isolates; one *S. mitis* isolate; and one *S. sanguis* isolate.

[b] Includes 12 *Escherichia coli*, 9 *Enterobacter cloacae*, 2 *Enterobacter aerogenes*, 1 *Enterobacter* sp., 2 *Klebsiella oxytoca*, 2 *Klebsiella pneumoniae*, 5 *Serratia marcescens*, 2 *Citrobacter freundii*, and 4 *Proteus mirabilis* isolates.

[c] GNB, gram-negative bacilli. Includes two *Pasteurella multocida*, one *Campylobacter jejuni*, four *Pseudomonas aeruginosa*, one *Pseudomonas putida*, one *Pseudomonas alcaligenes*, one *Flavimonas oryzihabitans*, two *Flavobacterium odoratum*, one *Haemophilus influenzae*, and one *Aeromonas* sp. isolate and one unidentified oxidase-positive, gram-negative bacillus.

[d] Includes nine *C. albicans*, four *C. tropicalis*, one *C. glabrata*, seven *C. parapsilosis*, and one *C. pseudotropicalis* isolate.

[e] NS, not significant ($P > 0.05$).

[f] NC, not calculated when total number of isolates was ≤10.

Among the significant variables which can affect the yield of blood cultures, it is generally agreed that the volume of blood cultured is the most important (7). For the BacT/Alert system, specifically, Weinstein et al. demonstrated a significant increase in yield between standard BacT/Alert bottles inoculated with 10 ml of blood, compared to 5 ml (14). One of the advantages of using blood culture bottles for the inoculation of body fluids other than blood is that the bottles are designed to culture 5 to 10 ml of blood (depending on the manufacturer and bottle type), far more than can effectively be cultured in, e.g., a thioglycolate broth tube.

Four types of blood culture bottles were used in this study, including standard aerobic and anaerobic and FAN aerobic and anaerobic bottles. Each FAN bottle recovered significantly more isolates than its standard counterpart, but there was no statistically significant difference in overall recovery between the FAN aerobic and FAN anaerobic bottles. As might have been anticipated, the FAN aerobic bottle recovered more yeast and the FAN anaerobic bottle recovered more anaerobic bacteria. The choice of a single bottle type may be influenced by specimen type as well as bottle type. For example, anaerobic isolates are much less common in synovial fluids than in peritoneal fluids. Nonetheless, if a single bottle is to be used, a

TABLE 3. Comparative yield of clinically significant isolates of bacteria and yeast from four bottle types

Microorganism(s)	Total no. of isolates	No. of isolates from:				P	
		Standard aerobic	FAN aerobic	Standard anaerobic	FAN anaerobic	Aerobic standard vs FAN aerobic	Anaerobic standard vs FAN anaerobic
Gram-positive cocci	138	100	125	109	127	<0.001	<0.001
S. aureus	51	36	50	38	50	<0.001	<0.001
Coagulase-negative staphylococcus	50	39	48	41	45	0.003	NS
Enterococci	18	11	12	14	15	NS[a]	NS
Streptococci	19	14	15	16	17	NS	NS
Gram-positive bacilli	4	4	3	2	2	NC[b]	NC
Gram-negative bacilli	54	44	46	39	45	NS	NS
Enterobacteriaceae	39	31	34	31	37	NS	0.03
Other GNB[c]	15	13	12	8	8	NS	NS
Anaerobes	9	1	1	5	9	NC	NC
Yeast	20	20	19	8	4	NS	NC
All microorganisms	225	169	194	163	187	<0.001	<0.001

[a] NS, not significant ($P > 0.05$).
[b] NC, not calculated when total number of isolates was ≤10.
[c] GNB, gram-negative bacilli.

FAN aerobic bottle seems best. In our experience, empiric antimicrobial therapy is more likely to lack coverage for yeast than coverage for anaerobes.

In this study, we demonstrated that significantly more isolates were recovered with the FAN bottles than with standard bottles or routine culture from both synovial and CAPD fluids. Of these two specimen types, CAPD fluids usually have the greater specimen volume, particularly when the collection bag is sent to the laboratory. We are aware of no studies which have specifically addressed the question of whether the number of significant isolates increases when more than 10 ml of a sterile body fluid other than blood is cultured in FAN bottles. Is there any incremental benefit to culturing 20 or 30 ml or more, such as is done with blood cultures? We attempted to analyze our data to provide that answer. Unfortunately, only about one-quarter of our CAPD fluids were submitted with sufficient volume to permit full (10-ml) inoculation of each bottle type.

Overall, the FAN bottles recovered about 17% more clinically significant isolates than either the standard bottles or the routine culture method used in this study. A legitimate question that was beyond the scope of this study is whether this increase in yield was meaningful to the management of the patients in the study. In their evaluation of the significance of the increased yield of FAN bottles compared with that for the standard BacT/Alert bottles for culture of blood, McDonald et al. concluded that the majority of isolates and septic episodes detected only by the FAN bottles, or only by the standard bottles, were clinically important (8). Intuitively, it makes sense to us that at least some of the increased yield in this study should be clinically significant, particularly for types of infections in which empiric therapy or duration of therapy is not always predictable.

In an evaluation of the clinical importance of isolates recovered only from broth cultures, Morris et al. concluded that the broth, inoculated as an adjunct to direct plating, seldom yields results that benefit patient management (9). Of the specimen types included in our study, Morris et al. now utilize a broth culture in their laboratory only for CAPD specimens (9). The use of broth-based systems or methods for CAPD specimens is generally well accepted (14).

Bobadilla et al. demonstrated the benefit of using blood culture bottles for culture of peritoneal fluid for patients suspected of having spontaneous bacterial peritonitis (SBP) (2). Indeed, at our institutions, blood culture bottles are routinely used for diagnosis of SBP. Specimens from patients suspected of having SBP were not included in this study, in part because bedside inoculation of blood culture bottles is routinely performed. However, given the increased yield which we have demonstrated in this study for the FAN bottles compared with the standard blood culture bottles, it seems prudent to use FAN bottles for this purpose.

Excepting specimens for diagnosis of SBP as well as the dialysate from patients undergoing continuous ambulatory peritoneal dialysis, is there a benefit to using blood culture bottles for culture of specimens from patients suspected of having routine peritonitis? Although the results of our study were not statistically significant, there was a trend favoring increased recovery from FAN bottles ($P = 0.09$). It is reasonable to postulate that with larger numbers of specimens, statistical significance might be achieved. Nonetheless, we believe that there are several arguments against the routine use of blood culture bottles for culture of peritoneal fluid. Of the specimen types included in this study, polymicrobic infections were seen most frequently with peritoneal specimens. The costs associated with subculturing multiple organisms on selective and nonselective aerobic and anaerobic media may not be justified. Perhaps, if a Gram stain were used to screen out specimens with multiple morphotypes, mixed cultures would not be as common. Another reason not to culture routine peritoneal specimens in blood culture bottles is because, in our experience, suspected cases of peritonitis are often treated with broad-spectrum antimicrobials targeted toward mixed aerobic and anaerobic infections. Complete identification of every potential bacterial pathogen is not always necessary.

There have been few published studies which examined the potential benefits of culturing synovial fluid in blood culture bottles. von Essen and Holtta reported that with a blood cul-

ture bottle, 21% of joint fluids that were negative by routine culture were positive (12). The percentage of false-negatives increased to 40% when the patients were receiving antibiotics at the time of specimen collection.

Yagupsky et al., who cultured joint fluids of children, reported that 10 of 11 isolates of *Kingella kingii* grew only from a Bactec blood culture bottle but not from a routine culture method (18). We did not recover any *K. kingii* isolates in this study. However, Yagupsky et al. cultured specimens from pediatric patients, whereas few of our synovial fluids were collected from children.

In our study, 72% of significant isolates from synovial fluid were recovered by the routine method, 78% were recovered in the standard blood culture bottles, and 100% were recovered in the FAN bottles. We believe that the increased sensitivity of the FAN bottles merits further study of the clinical utility of the increased yield. Treatment of septic arthritis is prolonged and, in our experience, often involves prosthesis infections. It is not unusual for us to have patients who have been partially treated at referring hospitals or patients who have been given preoperative antibiotics before cultures were obtained. The increased yield of the FAN bottles could be particularly beneficial for these types of patients.

The small number of amniotic and pericardial fluid specimens included in this study does not permit any meaningful conclusions to be made about culture of these specimens in BacT/Alert bottles.

We could not demonstrate any benefit from culturing pleural fluid in BacT/Alert bottles. The overall low yield as well as the lack of difference in results among the three culture methods leads us to advocate not using blood culture bottles for these specimens.

Another advantage of using BacT/Alert (or other similar continuous monitoring blood culture systems) for the culture of sterile body fluids other than blood may be to lower labor costs associated with processing and interpreting these specimens. Overall, in this study, about 75% of all specimens yielded negative results. With an automated system, such as BacT/Alert, linked to a laboratory information system, a combination of sensitivity and lower labor costs can be achieved. Again, this would not necessarily apply to all of the fluid types tested in this study, as we have already stated. This approach clearly has the potential for selective application.

In summary, the BacT/Alert system gave excellent results when used for the culture of sterile body fluids other than blood. The FAN bottles demonstrated superior recovery compared to either the standard bottles or routine culture. The extent to which the BacT/Alert system could be used as a replacement for or supplement to routine culture methods will be influenced by specimen type, patient population, institutional supply versus labor costs, and further analysis of the clinical utility of results produced by broth-based culture methods. Currently, two of our laboratories use aerobic FAN bottles for culture of synovial and CAPD fluids, while the third laboratory uses aerobic FAN bottles for all of the fluid types included in this study. Two of our laboratories use no plated media in addition to the FAN bottles, while the third laboratory uses one piece of plated media in addition to the FAN bottle.

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Controlled Clinical Laboratory Comparison of Two Supplemented Aerobic and Anaerobic Media Used in Automated Blood Culture Systems To Detect Bloodstream Infections

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A 20-ml blood sample was collected from adult patients with suspected bloodstream infections and distributed equally into the four volume-controlled bottles of a blood culture set consisting of aerobic and anaerobic BACTEC Plus/F bottles and aerobic and anaerobic BacT/Alert FAN bottles. All bottles were incubated in their respective instruments for a standard 5-day protocol or until the instruments signalled positivity. Samples in all bottles with negative results by these instruments were terminally subcultured. A total of 8,390 blood culture sets were obtained during the study period, of which 4,402 (52.5%) met the study criteria. Of these, 946 (21.5%) were positive either by instrument signal or by additional terminal subculture of all negative bottles and yielded growth of microorganisms. Five hundred eighty-nine (13.4%) blood culture sets were considered to have recovered 663 clinically significant organisms. When both the BACTEC and the BacT/Alert systems were used, 465 positive sets were detected; BACTEC alone detected 52 positive sets and BacT/Alert alone detected 72 ($P = 0.09$). No differences were found between the two systems in microbial recovery rate from blood cultures obtained from patients on antibiotic therapy. Significantly more members of the family *Enterobacteriaceae* ($P < 0.01$) were detected from patients without antimicrobial therapy by BacT/Alert than by BACTEC. The false-negative rates were 0.20% for BACTEC and 0.32% for BacT/Alert. A significantly higher false-positive rate was found for BACTEC ($P < 0.0001$). Both systems were comparable for the time to detection of microorganisms. However, gram-positive bacteria were detected faster by BACTEC and *Enterobacteriaceae* were detected faster on average by BacT/Alert. We concluded that both systems are comparable in their abilities to recover aerobic and anaerobic organisms from blood cultures and a terminal subculture might not be necessary for either of the two systems. The increased positivity rate when using an anaerobic bottle in a two-bottle blood culture set is due to the additional blood volume rather than to the use of an anaerobic medium.

Due to the high morbidity and mortality associated with bacteremia (7), the rapid detection and subsequent identification of microorganisms from blood remain critical services of the clinical microbiology laboratory. Numerous blood culture methods are available, and selecting the optimal system for the diagnostic laboratory is an important and often difficult task (15). Many remarkable improvements have been made in an attempt to reduce the time to isolate pathogens from blood. Advancements in the use of liquid media linked with automation technology have enhanced the ability of laboratories to provide faster blood culture results. Two continuously monitored noninvasive blood culture systems, the BACTEC 9240 (Becton Dickinson, Heidelberg, Germany) and the BacT/Alert (Organon Teknika, Eppelheim, Germany), are in widespread use. The main advantages of the two systems over previous generations of blood culture instruments include full automation once the bottles are loaded, a shorter time to detection of blood pathogens, considerable labor savings, and improved laboratory work flow. The BacT/Alert and the BACTEC 9240 systems monitor the growth of organisms by checking for elaboration of CO_2 with a colorimetric and a fluorescent sensor, respectively.

New aerobic and anaerobic media were developed by the manufacturers to remove a variety of growth inhibitors from patients' blood, to enhance the recovery of fastidious organisms, and to improve the detection of bacteremia and fungemia in patients receiving antimicrobial therapy. BACTEC Plus/F medium consists of soybean-casein digest broth, primary supplements, and two types of resin, a nonionic absorbing resin and a cation-exchange resin. FAN medium (BacT/Alert) is a brain heart infusion broth base containing Ecosorb, a proprietary substance that contains adsorbent charcoal, Fuller's earth, and other components.

A number of studies comparing the two systems have been carried out, but to our knowledge, none of them compared the supplemented aerobic and anaerobic BACTEC Plus/F media with the supplemented aerobic and anaerobic BacT/Alert FAN media in one controlled clinical trial.

This study was conducted to compare the performances of the two blood culture systems, BACTEC 9240 and BacT/Alert, in terms of microbial recovery, time to detection, and false-positive and false-negative rates, thereby indicating the performance of the aerobic and anaerobic BACTEC Plus/F resin media and the aerobic and anaerobic BacT/Alert FAN media.

MATERIALS AND METHODS

Patient population. This monocenter study was conducted from August 1995 through February 1997 in a 2,500-bed acute-care community hospital which provides a full range of medical and surgical care. The hospital cares for an appreciable number of transplant, immunosuppressed patients, as well as patients presenting common community-acquired infections. Blood cultures were obtained from all adult patients with suspected bacteremia during the study

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period and processed in the hospital-related Institute of Microbiology and Hygiene.

Study design. Blood culture sets were prepared for the purposes of the study; each set contained four volume-controlled and randomized bottles, one each containing aerobic and anaerobic BACTEC Plus/F media and aerobic and anaerobic BacT/Alert FAN media. Written instructions for obtaining a blood culture, the required amount of blood, and the order of inoculation of the bottles for clinicians were included. In respect of the patient and with the agreement of the ethical commission of our hospital, a 20-ml amount of blood for one blood culture set was considered the maximum to obtain from a single venipuncture. This amount was distributed equally into each of the four bottles. The inoculum volume was determined in the microbiology laboratory by weight. Sets in which any one of the bottles did not contain a minimum of 3 ml of blood or in which the volume difference between two bottles was more than 3 ml were considered noncompliant and excluded from the study but processed for the benefit of the patient.

On arrival in the laboratory, all bottles were placed in their respective instruments for a 5-day incubation time at 35°C and monitored in accordance with the manufacturers' recommendations. FAN aerobic bottles were vented before incubation.

Bottles indicated as positive by the instrument as well as bottles positive on arrival were Gram stained. Based on the Gram stain result, aliquots of the bottles were subcultured onto adequate media (chocolate agar incubated at 35°C in a 5% CO_2-enriched atmosphere or blood agar with aerobic and anaerobic incubation at 35°C). All microorganisms were identified by standard microbiologic procedures. All false-positive bottles (i.e., bottles that were smear and subculture negative after instrument signal) were replaced into the instrument during the 5 days of protocol. A terminal blind subculture onto chocolate agar and blood agar (aerobic and anaerobic incubation as described above) was performed for all negative bottles. False-negative bottles had no positive signal by the instrument, but growth occurred on the terminal subculture.

Statistical methods. The following data of all blood cultures were entered in a computer database: collection time, loading time, blood volume of each bottle, detection of growth by each bottle, growth on subculture and identity of microorganisms recovered, antimicrobic therapy, and the detection time for each positive bottle. Detection time of a microorganism was defined as the time between loading a blood culture bottle into the instrument and the time when a positive signal occurred followed by growth on subculture. For comparison of detection times, only clinically significant isolates recovered in both systems were taken into account. If both bottles of one system recovered the same organisms, the shorter time to detection was taken into account for comparison. The clinical importance of isolated microorganisms was determined after consultation with the patient's physician in accordance with published criteria (6, 10, 16, 18). Statistical analysis was done for blood cultures meeting the study criteria. Only isolates classified as being clinically significant were taken into account. Sensitivities were calculated in two manners. For comparison of the two systems, the common denominator of positive cultures was determined by all four terminal blind subcultures. For evaluating the usefulness of terminal subcultures in clinical routines, the detection rate of each system was determined with reference only to the related terminal subculture. The common denominator for the determination of both the false-positive and the false-negative rates for each system was the total of 4,402 compliant sets of the study. The main scientific goal of the study was the comparison of the sensitivities and specificities of both systems. For these two analyses, the Bonferroni correction was used. All other analysis on subgroups of patients or isolates must be considered explorative without protection against the error of multiple testing. All comparisons were evaluated by McNemar's test ($\alpha = 0.05$, two-sided) by using the statistical package SPSSWIN.

RESULTS

A total of 8,390 blood culture sets were obtained during the study period. Of these, 4,402 (52.5%) met the study criteria; of these, 946 (21.5%) yielded growth of 1,083 bacteria or fungi. Five hundred eighty-nine (13.4%) compliant sets, positive by either instrument signal or terminal subculture only, detected 663 bacteria or fungi that were considered clinically significant. Three hundred fifty-seven (8.1%) sets recovered only organisms classified as contaminants.

Of the 589 positive blood culture sets with significant isolates, 508 (86.3%) were positive by BACTEC 9240 and 523 (88.8%) sets were positive by BacT/Alert. BACTEC alone detected 52 (8.8%) positive sets, and BacT/Alert alone detected 72 (12.2%). With reference to the terminal blind subcultures of both systems, BACTEC and BacT/Alert showed sensitivity rates of 86.3 and 88.8% respectively. With reference to the system-related terminal subculture, the BACTEC instrument demonstrated a detection rate of 98.3% and the BacT/Alert instrument gave one of 97.4%.

TABLE 1. Comparative yields of clinically important bacteria and fungi in BACTEC Plus/F and FAN aerobic and anaerobic culture sets

Microorganism	No. of organisms detected by:			P
	BACTEC only	BacT/Alert only	Both systems	
Gram-positive bacteria	30	34	228	NS[a]
Staphylococcus aureus	11	14	124	
Staphylococcus epidermidis	2	2	9	
Viridans group streptococcus	2	5	18	
Enterococcus spp.	6	7	32	
Streptococcus pneumoniae	6	4	33	
Beta-hemolytic streptococci	3	1	9	
Listeria monocytogenes		1	3	
Enterobacteriaceae	21	45	187	<0.01
Escherichia coli	14	25	101	
Klebsiella spp.	3	12	27	
Enterobacter/Citrobacter spp.	2	5	31	
Salmonella spp.			6	
Proteus/Providencia/Morganella	1	1	11	
Serratia/Hafnia spp.	1	2	11	
Non-*Enterobacteriaceae*	18	13	36	NS
Haemophilus spp.		2	1	
Pseudomonas aeruginosa	7	7	8	
Stenotrophomonas maltophilia	3		6	
Neisseria meningitidis		1	1	
Acinetobacter spp.	8	3	18	
Other			2	
Anaerobic bacteria	6	3	4	NS
Clostridium perfringens	1	1	1	
Bacteroides spp.	3	2	2	
Candida spp.	5	6	14	NS
All microorganisms	80	101	482[b]	

[a] NS, not significant.
[b] For some species with a single isolate, data were not depicted separately but were included in the total.

Nine false-negative blood cultures occurred with the BACTEC instrument (three *Candida* species and single isolates of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Enterobacter agglomerans*, *Pseudomonas aeruginosa*, and *Pseudomonas stutzeri*) and 14 false negatives occurred with the BacT/Alert instrument (four *P. aeruginosa*, three *Acinetobacter* species, two *Stenotrophomonas maltophilia*, two *Candida* species, and single isolates of *Streptococcus sanguis*, *Escherichia coli*, and *Proteus mirabilis*).

The false-negative rates for the systems were 0.20% for BACTEC and 0.32% for BacT/Alert.

A significantly higher false-positive rate was found for BACTEC, 6.2%, than for BacT/Alert, 1.4% ($P < 0.0001$).

The comparative yields of bacteria and fungi from the two systems are indicated in Table 1. Of the 663 clinically significant organisms from compliant sets, 482 (72.7%) were recovered from both systems, 80 (12%) were recovered by BACTEC only, and 101 (15.2%) were recovered by BacT/Alert only. The differences observed in the recovery of bacteria reached statistical significance only for the *Enterobacteriaceae* group. The BacT/Alert system detected significantly more *Enterobacteriaceae* than the BACTEC system did ($P < 0.01$). A similar trend

TABLE 2. Comparative speed of detection of clinically significant organisms when both systems were positive and detection time was provided for both systems (n = 421)

Microorganism	Time to detection (h)		n	P
	BACTEC	BacT/Alert		
Staphylococcus aureus	8.4	11.2	124	<0.001
Viridans group streptococcus	4.0	6.6	18	<0.01
Enterococci	5.1	19.5	32	<0.05
Streptococcus pneumoniae	5.1	6.2	33	<0.05
Escherichia coli	7.6	6.9	101	<0.05
Klebsiella spp.	8.9	5.8	27	NS[a]
Enterobacter spp.	7.8	7.5	31	<0.05
Pseudomonas aeruginosa	15.4	20.7	8	NS
Acinetobacter spp.	6.9	10.2	18	<0.01
Mean values	9.0	10.4	482[b]	<0.05

[a] NS, not significant.
[b] For some species with fewer than 10 isolates, data were not depicted separately but were included in the mean time to detection.

was seen in the recovery of yeasts from blood cultures obtained from patients without antibiotic therapy. For all other organisms, there were no significant differences in the number of positive cultures between systems, neither by organism group nor by species.

Of all compliant blood culture sets, 41.9% (1,807 sets) were obtained from patients receiving antimicrobial therapy. Of the 589 positive compliant sets with significant isolates, 35.5% (208) were obtained from patients receiving antibiotics. There were no significant differences in isolation rates between the two systems for patients receiving antibiotics. The BacT/Alert system detected significantly more *Enterobacteriaceae* than the BACTEC system did ($P < 0.01$) from patients without antibiotic therapy.

The times to detection of several bacterial groups were compared for the two systems (Table 2). Gram-positive bacteria were detected significantly faster by the BACTEC system, whereas *Enterobacteriaceae* on average were recovered faster by the BacT/Alert system. There was no difference between the two systems in the cumulative percentage of positive cultures after 24, 48, and 72 h of incubation. Within the first 24 h, BACTEC detected 89.8% and BacT/Alert detected 89.6% of the positive cultures; after 48 h, 95.7 and 96.9% were detected by BACTEC and BacT/Alert, respectively; and after 72 h, 99.0 and 99.4% were detected by the two systems, respectively. The average times to detection were 9.0 h for the BACTEC system and 10.4 h for the BacT/Alert system ($P < 0.05$). The significant difference in average time to detection between both systems was caused by the higher detection rate within the first 6 h of the BACTEC instrument (50.7% detected by BACTEC and 45.8% by BacT/Alert).

Delayed entry was defined as a time difference of >12 h between blood sample collection and placement of a blood culture bottle into the respective instrument. More than half of the compliant positive blood cultures had a transportation time of >12 h due to the fact that our new hospital building is separated from the laboratory. Delayed entry had no effect on the sensitivity of the BacT/Alert instrument, but for the BACTEC system, the detection rate was significantly lower for blood cultures with delayed entry than for those with no delay ($P = 0.0097$). Differences also occurred in detection of the *Enterobacteriaceae* group: BacT/Alert recovered significantly more *Enterobacteriaceae* from blood cultures with delayed entry than BACTEC did ($P = 0.0062$) when the subgroup of patients not receiving antibiotic therapy was considered.

The data of the two systems allow not only a system-versus-system comparison but also a comparison of the two media, i.e., resin aerobic versus FAN aerobic media and resin anaerobic versus FAN anaerobic media (Table 3). No significant differences were seen between the aerobic media nor between the anaerobic media of the two systems in terms of detection rate. A comparison of the aerobic and anaerobic media of each system showed significant differences. The false-positive rate was significantly higher for both resin media than for the FAN media. The false-negative rates for both anaerobic media (4.7% for resin and 3.9% for FAN) were higher than those for the aerobic media (1.7% for resin and 2.2% for FAN).

Anaerobic bacteria causing septicemia were recovered from 13 compliant blood culture sets. Both systems detected four, the BACTEC system alone detected six, and the BacT/Alert system alone detected three anaerobic bacteria.

DISCUSSION

This monocenter-controlled evaluation compared the performances of the supplemented aerobic and anaerobic BACTEC resin media, under identical conditions of observation against the supplemented aerobic and anaerobic BacT/Alert FAN media in a clinical trial. Simultaneous inoculation of all four bottles from a single blood culture also enabled a system-versus-system comparison of the BACTEC 9240 and the BacT/Alert instruments. To our knowledge, no data have been published yet on this subject. In the past, several studies have evaluated the performance of the BACTEC (20) and BacT/Alert (2, 11) instruments, comparing either the two nonsupplemented media (19), the resin medium and the standard BacT/Alert medium (1, 9, 13), or the aerobic resin medium and the aerobic FAN medium (4, 8, 14). Outcomes have been quite different: the BACTEC system detected significantly more gram-positive cocci and the BacT/Alert system recovered significantly more *Enterobacteriaceae* in a first comparison (19) of the two nonsupplemented media.

Auckenthaler et al. (1) as well as Smith et al. (13) compared BACTEC resin medium to standard BacT/Alert medium, and both groups reported a better performance in terms of microbial recovery, false-positive rate, and detection time for the resin medium. A comparison of aerobic resin medium with aerobic FAN medium in a recent multicenter (4) study has demonstrated equal results. The previous single-center study by Pohlman et al. (8) showed a better recovery rate for *Entero-*

TABLE 3. Detection of positive cultures and false-positive signals by aerobic and anaerobic media

Medium	No. of positive cultures			No. of false-positive signals[b]
	Total	Detected by instrument[a]	Detected by terminal subculture	
Aerobic resin	466	456	10	185
Acrobic FAN	489	476	13	54
Anaerobic resin	448	420	28	116
Anaerobic FAN	429	406	23	11

[a] Values for aerobic resin versus aerobic FAN media and for anaerobic resin versus anaerobic FAN media are not significantly different. Values for aerobic resin versus anaerobic resin media and aerobic FAN versus anaerobic FAN media are significantly different ($P < 0.05$ and $P < 0.001$, respectively).
[b] Values for aerobic resin versus aerobic FAN media, anaerobic resin versus anaerobic FAN media, aerobic resin versus anaerobic resin media, and aerobic FAN versus anaerobic FAN media are significantly different ($P < 0.001$).

bacteriaceae and *Pseudomonas* by use of the BacT/Alert system with fewer false-positive results. In comparison with the results of other studies (4, 11, 12, 17), the positivity rate of blood cultures in our study was high (21.5% positive sets), with 13.4% sets recovering clinically significant organisms. With the exception of members of the *Enterobacteriaceae* group, which were recovered significantly ($P < 0.01$) more often by the BacT/Alert system alone, there were no significant differences in terms of microbial recovery between the two systems. The *Enterobacteriaceae* that were detected significantly more frequently by the BacT/Alert system resulted from blood cultures from patients without antimicrobial therapy. There was also a tendency towards a similar result for the fungi group: BacT/Alert detected slightly more *Candida* sp. isolates from patients without antibiotics than from patients with antimicrobial therapy. However, these are results of an explorative analysis in which, due to the small numbers of cases, a high beta error and the problems of multiple testing must be taken into account.

Reports of high false-negative rates, up to 6% (12, 13), suggest that terminal subculturing of negative BACTEC cultures after 5 days of incubation may be necessary, whereas others have suggested that this is not needed in the BacT/Alert system (3). In our trial, the false-negative rates were similarly low for both systems (0.20% for BACTEC and 0.32% for BacT/Alert) and the sensitivities for the BACTEC and BacT/Alert instruments with reference to the related terminal subcultures were 98.3 and 97.4%, respectively; therefore, we have concluded that a blind terminal subculture may not be necessary for either of the two systems.

The introduction of automated blood culture systems also serves the purpose of reducing the routine workload for laboratory personnel. A low false-positive rate is one important factor here. Our results showed a significantly ($P < 0.0001$) higher false-positive rate for the aerobic and anaerobic resin media (6.2%) than for the FAN media (1.4%). During the study period, both instruments were used in accordance with the recommendations and specifications of each manufacturer. Laboratory facilities were equal for both systems. All false-positive blood cultures were replaced into the instrument, incubated for the rest of the 5-day incubation period, and terminally subcultured. A small percentage of the false-positive signals by the BACTEC instrument occurred after an electricity breakdown, which did not affect the BacT/Alert instrument.

Due to the need to reduce laboratory costs, many microbiology laboratories do not have 24-h coverage by technical personnel. Especially in smaller hospitals, there is no microbiology laboratory available, so that blood cultures must be sent to external laboratories. Therefore, a delay may occur from the time blood is drawn until the bottle is placed into a blood culture instrument. Our hospital consists of an original building complex with 1,500 beds and a new building with 1,000 beds which is situated 10 km away. All blood specimens from the new building must be transferred to the laboratory in the original building, so that a delay of >12 h may occur.

Those blood cultures that were obtained after the microbiology laboratory was closed for the day were collected in a central laboratory that is open 24 h per day and were incubated at 35°C, so that almost all blood cultures with delayed entry were preincubated at 35°C. Delayed entry did affect the sensitivity of the BACTEC instrument in that the detection rate was significantly lower for blood cultures with delayed entry than for those with no delay ($P = 0.0097$).

The numbers of false-negative sets with delayed entry and of false negative sets with a transport time of <12 h were too small for valid comparison. For the BACTEC system, 6 of 9 false-negative sets were sets with delayed entry; for BacT/Alert, 8 of 14 false negatives were sets with delayed entry. In spite of the small number, there seems to be no need for blind terminal subculture either for sets with overnight preincubation at 35°C or for sets processed within the first 12 h after collection. Both systems detected organisms faster from culture sets with delayed entry since these sets were preincubated. With regards to the handling of preincubated blood culture bottles, the BacT/Alert system offers the advantage of a visible change on the bottom of a positive bottle so that the bottle can be processed onto subcultures before incubation into the instrument. The interpretation of Gram stain results for positive bottles was more difficult for FAN media than for resin media due to the charcoal particles in the media.

Staphylococcus epidermidis, which is considered a contaminant, was recovered more frequently from the FAN aerobic bottle ($n = 424$) than from the resin aerobic bottle ($n = 349$); this more frequent recovery may be due to the fact that the FAN aerobic bottle must be vented before incubation in the instrument. The use of needles to manually vent the aerobic FAN bottles may increase the probability of contaminating the bottle.

The question whether there is any advantage to using both aerobic and anaerobic blood culture media routinely (5) or whether anaerobic media should be used for the detection of bacteremia only in patients at risk of having an anaerobic infection is often raised. Only 13 positive blood cultures out of 589 sets with clinically significant organisms recovered anaerobic bacteria, 11 being isolated from the anaerobic medium only and 2 being isolated from both the aerobic and anaerobic media. Four of the 11 blood culture sets with obligate anaerobic bacteria represented polymicrobial septicemia; in addition to the anaerobic bacteria, *Enterobacteriaceae*, *Staphylococcus aureus*, and *Candida* spp. were isolated from the corresponding aerobic bottle as well. Definitively, seven (1.2%) blood cultures with monomicrobic obligate anaerobic pathogens would not have been detected by using only an aerobic bottle. By using the anaerobic resin bottle in addition to the aerobic one, the detection rate increased by 10.2%, with facultative anaerobic bacteria contributing 8.7% and obligate anaerobic bacteria contributing 1.6%. Analogously, the anaerobic FAN bottle increased the positivity by 9%, of which 8% was due to facultative anaerobic bacteria and 1% was due to strict anaerobic ones. Diagnoses of patients with septicemia due to obligate anaerobic bacteria included colitis ulcerosa, pancreatitis, diabetes mellitus with erysipelas, cholangitis, leukemia, and pneumonia, so that anaerobic infections could have been expected. According to Weinstein et al. (17), an increase in the volume of blood inoculated into BacT/Alert aerobic bottles (from 5 to 10 ml) increased the overall yield (7.2%) of clinically important organisms. From our results and those reported by Weinstein et al. (15, 17), we conclude that the higher recovery rate achieved when the anaerobic bottle is included is due to the inoculation of a larger blood volume rather than to the use of an additional anaerobic medium.

In summary, both systems are comparable for recovering clinically significant microorganisms from adult patients with bacteremia and fungemia receiving antibiotic therapy at the time of blood culture collection. With blood from patients without antibiotic therapy, the BacT/Alert system detected significantly more *Enterobacteriaceae*. The high false-positive rate of the BACTEC system caused additional work and material costs in our laboratory. With the BacT/Alert instrument, the software capabilities were more convenient for the user, and failure rarely occurred.

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Controlled Clinical Comparison of Three Commercial Blood Culture Systems

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Abstract:

In a controlled clinical comparison, three commercial blood culture systems – the standard aerobic BacT/Alert bottle (STD), the aerobic BacT/Alert FAN bottle (FAN) and the Isolator system (ISO; Wampole Laboratories, USA) were compared for their ability to detect aerobic and facultatively anaerobic microorganisms. A total of 945 BacT/Alert (STD and FAN) blood culture sets were compared. Of these, 110 blood culture sets (11.6%) yielded growth of 116 clinically significant bacterial and fungal isolates. Microorganisms were recovered from 10.7% (101/945) of the FAN bottles compared to 8.9% (84/945) of the STD bottles. Of the significant isolates, 78 (67.2%) were recovered by both bottles, 29 (25%) by the FAN bottle only and nine (7.8%) by the STD bottle only ($P<0.01$). Along with 56.1% (530/945) of BacT/Alert blood culture sets, a concomitant ISO tube was obtained. Of the triple (STD+FAN+ISO) blood culture sets, 54 (10.2%) yielded growth of 59 clinically relevant isolates. Microorganisms were detected in 9.1% (48/530) of the FAN bottles, 8.3% (44/530) of the STD bottles and 4% (21/530) of the ISO tubes ($P<0.001$). Overall, the BacT/Alert system detected more clinically significant microorganisms than the ISO tube; the STD and the FAN bottle each recovered significantly more staphylococci ($P<0.01$ and $P<0.001$, respectively) and gram-negative rods ($P<0.01$, both). In conclusion, the BacT/Alert FAN bottle performed better than the BacT/Alert STD bottle; both BacT/Alert bottles, however, were superior to the ISO tube in terms of recovery of clinically significant microorganisms, including gram-positive and gram-negative bacteria.

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A Cell Viability Assay Based on Monitoring Respiration by Optical Oxygen Sensing

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A cell viability assay based on monitoring of the metabolic activity of living cells via their consumption of dissolved oxygen has been developed. It uses a microwell plate format and disposable phosphorescent sensor inserts incorporated into each sample. The wells are subsequently sealed from ambient oxygen using a layer of mineral oil, and periodically scanned from underneath with a simple fiber-optic phosphorescent phase detector. Thus, dissolved oxygen levels and time profiles of cell respiration can be determined noninvasively and compared to each other. The system was tested by monitoring the viability of the fission yeast *Schizosaccharomyces pombe*. In comparison with the conventional cell densitometry assay, the optical oxygen sensor method could reliably monitor lower numbers of cells (10^4–10^5 vs 10^6–10^7 cells/ml for densitometry), and accurately determine culture viability within 1 h. The assay was then applied to determine the viability of samples treated with toxic agents such as azide and in response to expression of a physiological inducer of cell death, the Bcl-2 family member Bak. The results obtained confirm that measurement of cell respiration by this assay can serve as a predictable, reliable, and fast method for high-throughput determination of cell viability and growth.

Key Words: **cell viability assay; optical oxygen sensor; cell respiration; phosphorescent porphyrin probe; apoptosis; bak.**

Many biological assays are based on determining the viability or activity of living organisms. Such methods usually involve application of a biological sample into an appropriated growth medium followed by monitoring of certain parameters reflecting the activity of organism over time. These parameters include cell concentration, metabolic activity, activity of particular enzymes, and morphological properties. These approaches are widely used, for example, in cell cytotoxicity tests, in drug discovery for agents such as chemotherapeutic drugs, in studies of cell proliferation or apoptosis, in screening of microorganism strains, optimization of fermentation conditions, in sterility tests. Conventional methods used for determination of cell viability or growth include cell densitometry, microscopy in combination with various staining techniques, as well as metabolite assays (1–6). Cell growth of yeast or bacteria can be monitored by measuring the absorbance of samples at about 600 nm which increases proportionally to the concentration of cells (2). This method is time-consuming and lacks sensitivity, and it is limited by the cell division cycle time.

The problem of long assay times can be overcome by monitoring cellular metabolic activity, which in many cases can give a more rapid and sensitive response. The measurement of the amount of ATP by bioluminescence is sensitive (as low as 20 cells/ml) and has a wide linear range (up to 10^7 cells/ml) (3, 4). The MTT assay is based on the intracellular reduction of a tetrazolium dye to a blue formazan product measured spectrophotometrically and is used for high-throughput screening (5). It has lower sensitivity (limit of detection approximately 2.5×10^4 cells/ml), linear range (one order) than the ATP assay, and pH dependence (6). The BACTEC assay measures the cellular conversion of glucose to carbon dioxide and is based on measuring carbohydrate metabolism as a marker of cell viability (7). It utilizes fluorescent labels or radioisotope (^{14}C)

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and has been applied to high-throughput microbiological screening [8]. However, all of these methods are unsuitable for continuous monitoring. To obtain cell growth/viability curves periodic sampling is required.

Oxygen is one of the key metabolites in aerobic systems and the rate of oxygen uptake is a good indicator of metabolic activity of cells which can be correlated to viability. This approach usually requires sealed vials or test cells to exclude vast excess of oxygen in ambient air. The Clark-type oxygen electrode is commonly used to monitor respiration of cells (9), but it has certain disadvantages such as consumption of oxygen, drift of calibration, poisoning by certain chemical species, invasiveness, and problems with sterilization. Measurement of pressure change in a sample headspace using a built-in pressure sensor is another technique that is used in tests for biochemical oxygen demand (BOD_5)[2] (10). This method is not very sensitive, it is time-consuming due to the high oxygen capacity of test vials, and it is not suited to small sample volumes. Both techniques require rather costly devices for each individual sample, and are therefore unsuitable for the processing of large groups of samples.

The optical oxygen sensor approach offers a convenient alternative to the above methods. The principle employed is to exploit changes in fluorescence of a solid-state probe in response to changes in oxygen concentration (11, 12). Probes based on fluorescent complexes of ruthenium (13, 14) and phosphorescent porphyrin dyes (15, 16) embedded in appropriated oxygen-permeable polymers are commonly used as sensing materials. Quantitation of oxygen via quenching of fluorescence can be performed by either the intensity or lifetime measurements (11, 12, 17, 18). As discussed elsewhere (19–21), lifetime-based systems are generally considered superior due to their independence of dye concentration, optical geometry, sensor positioning, luminophore degradation, light source, and detector fluctuations and aging. Employing phase-modulation techniques, these systems require rather simple and inexpensive equipment suitable for real-time continuous monitoring of oxygen (16, 18, 22, 23). One of the advantages of fluorescence-based oxygen sensors is the possibility of noninvasive sensing through a transparent vessel because only *optical* contact between the probe and the luminescent detector is required (11, 20, 24, 25). Disposable sensor elements having predetermined calibration are simple, cheap, robust, and suitable for "contactless" measurements on a microscale.

In the present paper the phosphorescence-lifetime-based oxygen sensor (16, 26) has been applied to develop a simple assay for cell viability. The system deploys solid-state oxygen sensor inserts and contactless monitoring of respiration profiles of aerobic cells using a fiber-optic phosphorescence phase detector. The sensor responds to oxygen concentration by changing its phosphorescence lifetime, which is a self-referencing parameter that does not require calibration and that is monitored by a phase detector. Thus, profiles of oxygen consumption (initial slopes or whole curves) can be obtained as a time trace of increasing phosphorescence phase shift signal, and then compared under different conditions. The performance of the system was optimized, compared to conventional methods, and employed to measure yeast cell viability and growth. We show that it can be reliably used to monitor the viability of yeast cells in response to different toxic and physiological stimuli.

EXPERIMENTAL

Preparation of the phosphorescent oxygen-sensitive membrane. A coating "cocktail" was prepared which contained 0.5 mg/ml of platinum(II) complex of octaethylporphine-ketone (PtOEPK, Joanneum Research, Graz, Austria) in 5% (w/w) solution of polystyrene (M_r 230,000, Aldrich) in ethyl acetate. Aliquots of 2 μl of this solution were applied with a micropipet onto pieces of 0.22-μm pore size Durapore filter membranes (Millipore) and allowed to spread and air-dry for approximately 15 min. Thus, uniformly colored pink spots having a diameter of about 5 mm were obtained which were cut into disks of the same diameter, phosphorescent oxygen sensor membrane inserts. These were stored in dark place for further use.

Yeast culture and modulation of yeast cell viability. *Schizosaccharomyces pombe* cells (derived from wild-type strains $972h^-$ and $975h^+$) were maintained on complete medium plates. For isolation in logarithmic growth phase small plated colonies were removed and transferred to 10 ml YES complete growth medium (2 g casamino acids, 30 g dextrose, 5 g yeast extract in 1 liter demonized H_2O) and allowed to equilibrate at 30°C for approximately 8–10 h. Samples were removed periodically for determination of cell number using a hemocytometer or measurement of optical density at 600 nm. To determine the affects of azide on yeast oxygen consumption and viability, sodium azide at different concentrations was added to an exponentially growing yeast culture.

A strain of *S. pombe* with stable integration of the *bak* gene (27) under the control of the nmt promoter was obtained from G. Evan (ICRF, London). These cells were grown to mid-logarithmic phases in 10 ml minimal medium (EMM) containing thiamine to repress bak expression (15 μM). To induce bak protein expression, cells were washed and cultured in thiamine-free

[2] Abbreviations used: BOD, biochemical oxygen demand; EMM, minimal medium; PtOEPK, octaethylporphine-ketone; YES complete growth medium, 2 g casamino acids, 30 g dextrose, 5 g yeast extract in 1 liter demonized H_2O.

EMM, and cultures with either repressed bak or induced bak were maintained in logarithmic phase growth for 27 hours post induction. Western blotting confirmed that bak protein expression was visible in the induced cultures at 16 hours. Viability assays were performed between time 14 h and 27 h post induction.

Apparatus. Construction and principles of operation of the phosphorescence phase detector—the main measurement block of the optical oxygen sensor—are described in detail elsewhere (16, 26). The device is equipped with a 60-cm fiber-optic probe (5 mm diameter) and enables real-time continuous monitoring of the phosphorescence intensity and phase shift signals from PtOEK-PS sensors, and temperature using a built-in T-probe. In this study a working frequency of 2605 Hz was used as a standard. Monitoring of these primary parameters makes it possible to calculate the sample oxygen partial pressure (in kPa), using the predetermined analytical relationships. This presumes a detailed characterization of disposable sensor elements prior to their use and correction for variations of sample temperature (26). The fiber-optic probe enables remote and contactless sensing experiments and facilitates manipulations with samples and sensor inserts. The instrument is connected to a PC via serial interface and operates by means of Windows-compatible software. The latter enables external control of the instrument, simultaneous recording of all related parameters such as phase shift, intensity, and temperature, graphical presentation, storage, and processing of measured parameters. The instrument is now produced on a small scale at Moscow Power Engineering Institute (Russia).

A special accessory for monitoring respiration of cells in a microwell strips was constructed. The main part of this accessory is an aluminum holder connected to an external circulation bath (temperature control). The strip was placed in a holder in a special rail (1 cm deep) which enabled bidirectional movement of the strip to be performed manually. The fiber-optic probe of the phase detector was attached to the holder, so as to face the bottom of the strip and allow measurements in one of the wells. Measurement setup is shown schematically in Fig. 1.

Measurement procedure. A typical experiment in a 8-well flat-bottom strips (Nunc) was performed as follows. Oxygen sensor inserts were placed into each well and growth medium was added (typically 0.2 ml per well) with a micropipet and allowed to come to temperature equilibrium (~10–15 min). In certain cases growth media contained various concentrations of effectors such as azide. Small aliquots (0.02 ml) of stock solution of yeast cells (10^6–10^7/ml) were added and then 0.1 ml of heavy mineral oil (Sigma) was applied to each well, and monitoring of the phosphorescent phase shift signal was initiated starting from the first well. Each well was monitored for 2–5 min and then the strip was moved so as to monitor the next well, and so on. One experiment usually took 30–120 min and included 5–10 measurement cycles for each well. The instrument software plotted time profiles of the phase shift for each well and stored measured data as spreadsheet files. The phase shift profiles can be converted into oxygen concentration scale (kPa). All measurements were carried out under ambient light without special precautions and at ambient temperatures (22–25°C).

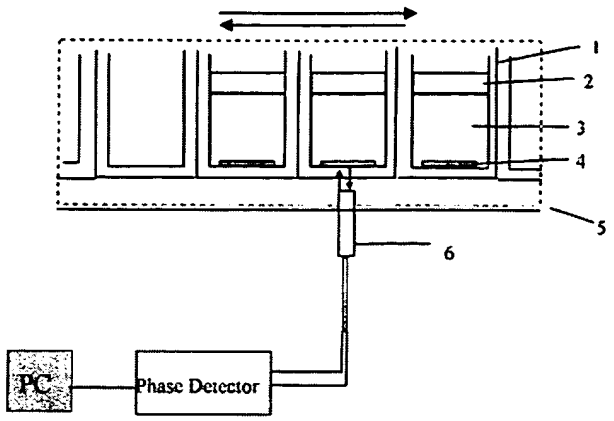

FIG. 1. Diagram showing the experimental setup for measurement of dissolved oxygen profiles. 1, 8-microwell strip; 2, mineral oil exclusion layer; 3, growth medium; 4, microporous membrane filter with oxygen-sensitive PtOEPK/PS coating; 5, thermostated holder; 6, scanning fiber-optic probe.

RESULTS AND DISCUSSION

Measurement setup and assay optimization. The oxygen-sensitive probe is the previously optimized polymer composition of the phosphorescent dye PtOEPK and polystyrene applied on a microporous membrane filter (26). In the setup described above, with the sensors cut into small inserts, the system displayed satisfactory performance, with respect to the stability, reproducibility of the measured signal, and sensitivity toward small changes of oxygen concentration. The response time (t_{90}) of the system on addition of sodium sulfite (complete chemical deoxygenation of solution) was 3.92 ± 0.52 min in PBS and 4.3 ± 0.55 min in the yeast growth medium YES. As the cells consume oxygen by respiration, the lifetime of the phosphorescent probe increases, and so does the phase shift. The relationships between the phosphorescence lifetime, phase shift, and oxygen concentration are discussed in details elsewhere (11, 18). In a simplified form they can be presented by the two equations

$$tan(\phi) = 2 * \pi * \nu * \tau, \quad [1]$$

$$\tau_0/\tau = 1 + k_q * \tau_0 * [O_2], \quad [2]$$

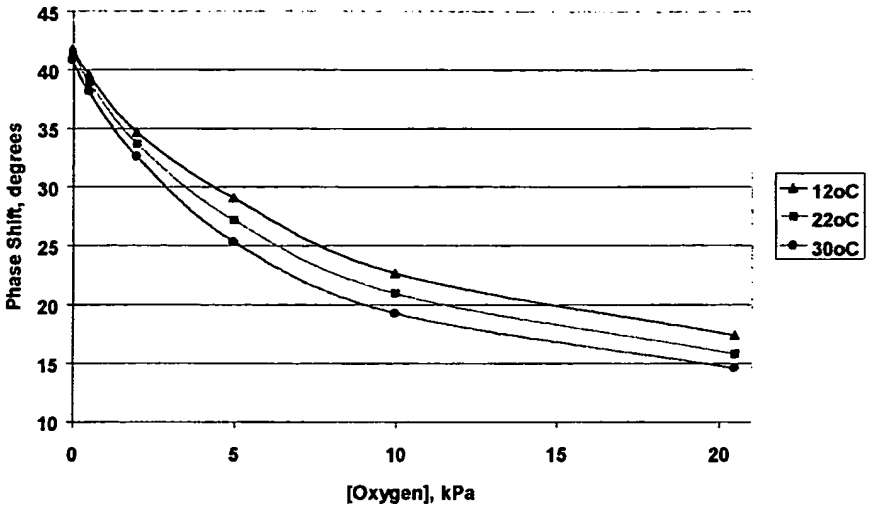

FIG. 2. Experimental relationship between the measured phase shift and sample oxygen concentrations (calibration graphs) for the disposable oxygen sensor inserts, measured at different temperatures.

where ϕ is the measured phase shift (degrees angle); ν is the instrument working frequency (Hz); τ and τ_0 –are the phosphorescence lifetime(s) in the presence and in the absence of oxygen, respectively; k_q –is the bimolecular quenching rate constant; $[O_2]$ is the oxygen concentration (kPa); and $\pi = 3.14$.

Typical calibration curves for a batch of disposable oxygen sensor inserts measured in aqueous solution at different temperatures are shown in Fig. 2. Typical variation in the measured parameters between individual sensors is usually under 1% (RSD). When monitoring small oxygen gradients in air-equilibrated aqueous samples (~21 kPa oxygen), the relationship between measured phase shift and oxygen concentration is close to linear (see graph).

The measurement of respiration profiles in small samples demands exclusion of ambient air which otherwise can cause interference due to vast excess of oxygen. Although it was possible to observe oxygen gradients in samples containing growing yeast cells in strip wells which were directly exposed to ambient air, it was found that the addition of a layer of mineral oil on top of the sample resulted in enhanced sensitivity and reliability of measurements. The effect of mineral oil seal was therefore investigated in detail to optimize the assay performance.

Profiles of the dissolved oxygen measured with the oxygen sensor inserts in samples which were degassed with helium and then left to rest are shown in Fig. 3. In the presence of the mineral oil seal (which has high viscosity and serves as a good barrier for air oxygen) the curve is much smoother, especially when approaching the ambient (i.e., air-saturated) oxygen range (the initial signal level). In the presence of the oil layer it takes the system many hours to come back to gas equilibrium. This means that minor consumption of the dissolved oxygen in air-equilibrated sample (i.e., reverse process) caused by respiration of living cells will result in higher gradients inside the sample in the presence of oil seal, and can be easily detected. An additional function of the oil seal in this type of assay would be to prevent evaporation and contamination during long assay times. Since oil is more convenient than other mechanical seals such as plugs or films, it was used in all subsequent experiments.

To optimize the assay performance and achieve maximal sensitivity of the system, volumes of sample and mineral oil were varied using cultures of yeast cells that were at a constant concentration, and the initial slopes of the phase shift were measured. Data are shown in Table 1. It is clear that the addition of oil increased oxygen gradients by more than threefold (Experiments 1 and 2). There is a clear effect of the sample volume on the measured phase shift slope (Experiments 2–5), which could be explained by the precipitation of the cells and creation of local oxygen gra-

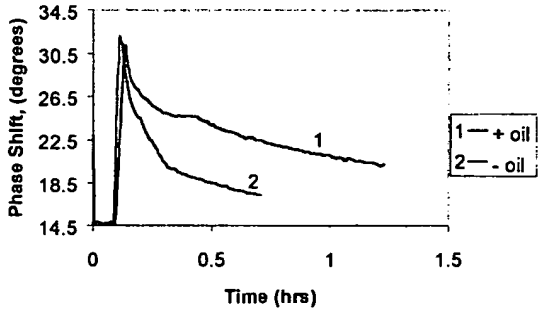

FIG. 3. The effect of mineral oil as an exclusion layer for ambient oxygen. Samples containing oxygen sensor inserts and growth media (without cells) were purged with He and then oxygen diffusion was monitored over time, at 22°C.

TABLE 1

Effect of the Sample and Exclusion Layer Volumes on the Optical Response

Experiment	Volume of medium, μl	Volume of oil, μl	Phase shift slope, °/h
1	200	0	2.78
2	**200**	**100**	**8.19**
3	150	100	5.92
4	100	100	3.96
5	50	100	0.97
6	200	150	7.87
7	200	50	8.26

Note. *S. pombe* cells at concentrations of 1×10^6 cells/ml were monitored at 22°C.

dients at the sensor membrane because no shaking was used. Therefore, in such an assay format the total amount of cells in a sample is an important factor. A small dependence on the amount of oil added was found in Experiments 2, 6, and 7, suggesting that even 50 μl of oil is sufficient to seal the sample from air. Based on this study, the conditions used in Experiment 2 were chosen for further experiments.

Monitoring respiration of S. pombe yeast cells. To apply the assay to measurement of yeast cell viability and growth, we first determined the respiration profiles of yeast cultures containing different initial concentrations of cells (indicated) in microwells measured with an oil insulation layer (Fig. 4). *S. pombe* cells have a doubling time of 3–5 h under these culture conditions and from a starting density of approximately 10^6 cells/ml can grow exponentially until they reach 10^7 cells/ml. Oxygen consumption was monitored in cultures diluted to contain 1×10^5 to 1.76×10^6 cells/ml over a 2-h time period, and displayed good reproducibility with coefficients of variation for identical samples within 4% ($n = 4$). As can be seen in Fig. 4, a clear differentiation between samples with different concentrations of cells can be achieved within 10 min. At concentration of 1.76×10^6 cells/ml almost complete depletion of sample oxygen is achieved within 20–30 min and the phase signal levels off. A phase shift of about 43° corresponds to zero oxygen concentration (Fig. 2).

For quantitative comparison of metabolic activity between different samples, the initial slope of the phase shift is sufficient as a measure of the metabolic activity (or viability) of living cells. For *S. pombe* yeast cells this parameter can be reliably determined over a period of 10–20 min. In a typical cell densitometry assay performed with *S. pombe* cells in standard glass flasks at 30°C, concentrations of 10^6–10^7 cells/ml and measurement times of 24 h are usually required. At room temperature and in microwell plates such assays would be even slower.

Significantly, the data obtained with oxygen consumption measurements were predictive of growth of *S. pombe* cells seeded at the different concentrations. Cultures seeded at $1–2 \times 10^5$ cells/ml do not normally grow in culture and this was reflected in the oxygen consumption profile, which was flat. Cells seeded in the region of 10^6 cells/ml grow optimally and this was again reflected in the curve obtained with cells seeded at 8×10^5/ml, which consistently produced the most linear curve. Therefore, we can conclude that this assay can monitor the growth of yeast cells, provided they are seeded at an optimal starting density (approximately 10^6 cells/ml).

Evaluation of viability of S. pombe cells in response to toxin and induction of cell death. Initial experiments demonstrated that the oxygen consumption assay could be used to measure live and proliferating cells. The next objective was to test whether the assay could discriminate accurately and rapidly between live cells and cells that had been poisoned with a toxin or cells that had been induced to undergo cell death. To poison cells we used sodium azide, a chemical toxin which inhibits the key enzymes of the respiratory chain, and measured a dose–response effect on oxygen consumption. Respiration profiles of *S. pombe* cells cultured in the presence of different concentrations of azide and measured with the optical oxygen sensor are shown in Fig. 5. They demonstrate that azide instantly inhibits the respiration activity of cells even at very low concentrations. Very pronounced differences in respiration profiles suggest rapid cell death in the azide-treated samples. This was confirmed by leaving samples of the yeast cells that had been treated in culture, and observing that they died.

We next tested a physiological regulator of cell death bak, which is a pro-apoptotic member of the bcl-2 family (27). In *S. pombe* cells stably expressing the bak gene under the control of the nmt promoter, induction

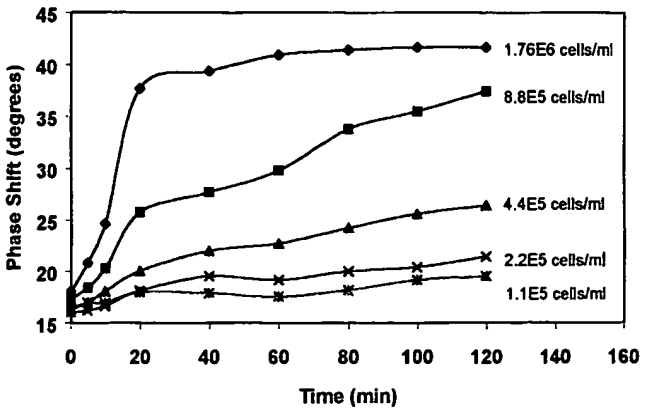

FIG. 4. Respiration profiles of different concentrations of yeast fission cells *S. pombe*, 22°C.

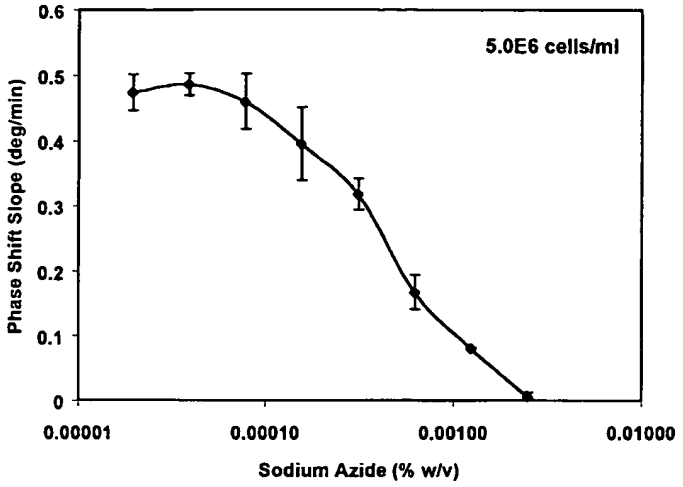

FIG. 5. Respiration activity of fission yeast *S. pombe* (5×10^6 cells/ml) with different concentrations of added sodium azide, monitored with the optical oxygen sensor. Each point represents an average value of four identical samples ($n = 4$) measured in parallel.

of bak expression has been shown to induce cell death with the characteristics of apoptosis seen in mammalian cells (28). Normally, to measure the bak phenotype in *S. pombe*, protein expression is induced by removal of thiamine from the culture medium (29) and the growth of the induced yeast is compared with a matched uninduced control by densitometry over 30 h. To determine whether the optical oxygen sensor could measure induction of cell death by bak, two cultures were monitored over time: one that had bak expression induced and the matching culture where bak expression was repressed. The results are shown in Fig. 6A and are compared with densitometric measurement of similar cultures in Fig. 6B. As can be seen in Fig. 6A, respiration activity of cells grown in thi^+ and thi^- medium, presented as the initial slope of the phase shift vs cultivation time, shows clear differentiation very quickly at 16 h. This is only 4 h after protein induction. When there is no induction of apoptosis (thi^+ uninduced sample), the cells remain in the exponential phase of growth, and the measured slope of the phase shift continuously increases. However, in the cells which had bak expressed (thi^-) the consumption of oxygen is substantially inhibited. The effect increases with time, demonstrating cell death of the bak repressed sample. These results obtained with the oxygen sensor were in very good agreement with those measured by conventional cell densitometry (Fig. 6B) and suggest that the oxygen sensor could discriminate between the live culture and the culture expressing bak, earlier than densitometry could. These results demonstrate that the optical oxygen sensor approach can be used to measure physiological regulators of cell death/viability, and can give a more rapid readout of cell viability than the conventionally used growth curves based on densitometry.

CONCLUSIONS

The cell viability assay based on the optical oxygen sensor approach was shown to be a realistic alternative to conventional methods and techniques. The mineral oil exclusion layer placed on top of each sample provided a convenient way to seal it from ambient air, and thus achieve high and reproducible oxygen gradients in small samples. A simple microassay format was developed and optimized which enables one to perform evaluation of multiple samples in parallel in microwell plates or strips using a simple instrument—phosphorescent phase detector with scanning fiber-optic probe, and disposable oxygen sensor inserts placed in each sample. In contrast to other methods, this system is

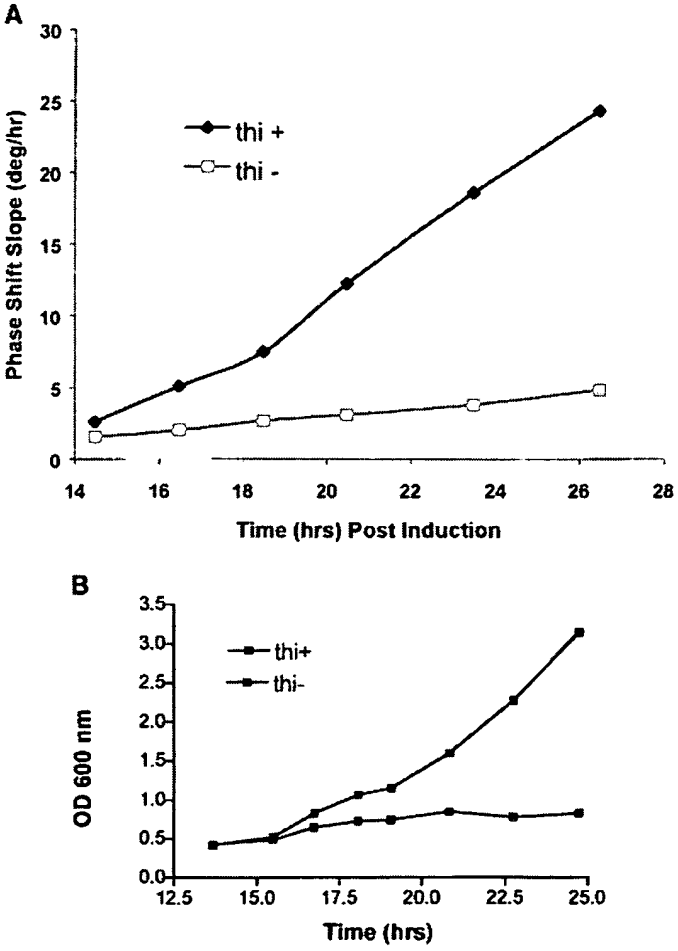

FIG. 6. Viability assay of fission yeast *S. pombe* with stably integrated human *Bak* cDNA. Effect depressed in the presence of thiamine and repressed in thi^- medium: A, respiration activity monitored with the optical oxygen sensor; B, cell growth monitored by densitometry. Cell death was observed in thi^- sample after 15 h incubation at 30°C.

capable of monitoring respiration profiles of aerobic cells in both a nonconsumptive and noninvasive manner. Such a simple, rapid, and sensitive assay is well suited to screening large numbers of samples. For high-throughput screening applications, automation of the measurement system is a realistic possibility, and it can be easily adjusted to conventional microwell plates.

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Arch Microbiol. 1978 Jan 23;116(1):61-7. Related Articles, Links

The alternate respiratory pathway of Candida albicans.

Shepherd MG, Chin CM, Sullivan PA.

Candida albicans contains a cryptic cyanide and antimycin A insensitive respiratory system. This alternate oxidase was found (i) at all growth rates from mu = 0.05 to 0.26 in a chemostat culture and (ii) in both mycelial and yeast forms of the organism. Neither chloramphenicol nor cycloheximide prevented the expression of the alternate oxidase. Salicylhydroxamic acid was a potent inhibitor of the cyanide insensitive respiration. The respiration of mitochondria grown in the presence of antimycin A was not inhibited by cyanide or antimycin A but was inhibited by salicylhydroxamic acid.

PMID: 203238 [PubMed - indexed for MEDLINE]

Biochim Biophys Acta. 1981 Jan 14;634(1):11-8.

Related Articles, Links

Evidence for a plasma membrane redox system on intact ascites tumor cells with different metastatic capacity.

Cherry JM, Mackellar W, Morre DJ, Crane FL, Jacobsen LB, Schirrmacher V.

A NADH-ferricyanide reductase of the external surface of intact mouse ascites tumor cells grown in culture was shown. The oxidation/reduction reaction was due to enzymatic rather than inorganic iron catalysis as demonstrated by the kinetics and specificity of the reaction. Activities of three markers for cytoplasmic contents were lacking with the intact tumor cells. The dehydrogenase activity was inhibited by p-chloromercuribenzoate, bathophenanthroline sulfonate, and the anticancer drug adriamycin. Sodium azide and potassium cyanide inhibited partially. The response to inhibitors resembled that of isolated plasma membranes rather than that of mitochondria. Concurrent with these findings, neither superoxide dismutase nor rotenone affected the redox activity. The findings provide evidence for the operation of a plasma membrane redox system at the surface of intact, living cells.

Fundam Appl Toxicol. 1991 Apr;16(3):435-48. Related Articles, Links

Assessment of mitochondrial membrane potential as an indicator of cytotoxicity.

Rahn CA, Bombick DW, Doolittle DJ.

Cellular/Molecular Biology Division, R. J. Reynolds Tobacco Company, Winston-Salem, North Carolina 27102.

Mechanistically based short-term in vitro tests to evaluate the relative cytotoxicity of of chemicals will complement in vitro genotoxicity testing during the initial phases of toxicity evaluation as well as provide information on the cellular site of action for chemicals found to be toxic in animals. The objective of this study was to characterize a procedure for evaluating mitochondrial membrane potential, an integral component of cellular energy homeostasis and normal cellular function, as an in vitro indicator of chemically induced cytotoxicity. Rhodamine 123, a cationic fluorescent dye whose mitochondrial fluorescence intensity decreases quantitatively in response to dissipation of mitochondrial transmembrane potential, was used to evaluate disturbances in mitochondrial membrane potential. Cultured rat liver epithelial cells (WB cell line) or human skin fibroblasts (MSU-2 cell line) treated with the oxidative phosphorylation uncoupler 2,4-dinitrophenol (DNP) or the cytochrome oxidase inhibitor sodium azide were used to characterize the system. In addition, acetaldehyde, which has been reported to damage the plasma membrane, but not the mitochondrial membrane, was used to demonstrate the specificity of this assay system. Mitochondrial membrane potential was not significantly affected by the cell culture density, as long as the cells were in the logarithmic phase of growth. The stage of the cell cycle influenced the mitochondrial membrane potential in human skin fibroblasts (highest in late G1-early S) but not in rat liver cells. DNP and sodium azide significantly (p less than 0.01) reduced the mitochondrial membrane potential in both cell lines compared to untreated cells, while acetaldehyde did not reduce the mitochondrial membrane potential in either cell line. This assay provides a tool for evaluating the effect of chemical treatments on mitochondrial membrane potential, as well as an indicator of cytotoxicity which does not require the use of animals.

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SYSTEM:OS  - DIALOG OneSearch
  File 349:PCT FULLTEXT 1979-2005/UB=20050331,UT=20050324
         (c) 2005 WIPO/Univentio
  File 654:US Pat.Full. 1976-2005/Mar 31
         (c) Format only 2005 The Dialog Corp.

      Set  Items  Description
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? exs
Executing TG22807312
HILIGHT set on as '%'
               8  OXYDISH?
              61  OXYRASE?
            5187  NAN3
           60083  AZIDE?
               0  SODIUM-AZIDE?
      S1       2  (OXYDISH? OR OXYRASE?) (100N) (NAN3 OR AZIDE? OR
                  SODIUM-AZIDE?)
? t s2/3/all
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? t s1/3/all

 1/3/1     (Item 1 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT
(c) 2005 WIPO/Univentio. All rts. reserv.

01011698
A  MEDIUM  COMPOSITION,  METHOD  AND  DEVICE  FOR SELECTIVELY ENHANCING THE
    ISOLATION  OF ANAEROBIC MICROORGANISMS CONTAINED IN A MIXED SAMPLE WITH
    FACULTATIVE MICROORGANISMS
COMPOSITION  DE  MILIEU,  PROCEDE  ET  DISPOSITIF PERMETTANT D'AUGMENTER DE
    MANIERE  SELECTIVE  L'ISOLEMENT DE MICRO-ORGANISMES ANAEROBIES CONTENUS
    DANS UN ECHANTILLON MELANGE PRESENTANT DES MICRO-ORGANISMES FACULTATIFS
Patent Applicant/Assignee:
  OXYRASE INC, P.O. Box 1345, Mansfield, OH 44901, US, US (Residence), US
    (Nationality)
Inventor(s):
  COPELAND James C, 298 N. Countryside Drive, Ashland, OH 44805, US,
  MYERS Kathy J, 269 Bowland Street, Mansfield, OH 44907, US,
Legal Representative:
  KLEIN Richard M (agent), Fay, Sharpe, Fagan, Minnich & McKee LLP, 1100
    Superior Avenue, Seventh Floor, Cleveland, OH 44114-2579, US,
Patent and Priority Information (Country, Number, Date):
  Patent:               WO 200340285 A1 20030515 (WO 0340285)
  Application:          WO 2002US16677 20020520  (PCT/WO US0216677)
  Priority Application: US 20017739 20011108
Designated States:
(Protection type is "patent" unless otherwise stated - for applications
prior to 2004)
  AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ
  EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR
  LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI
  SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
  (EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
  (OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
  (AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW
  (EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 11288


 1/3/2     (Item 1 from file: 654)
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DIALOG(R)File 654:US Pat.Full.

0005305261 **IMAGE Available
Derwent Accession: 2004-051087
Medium composition, method and device for selectively enhancing the isolation of anaerobic microorganisms contained in a mixed sample with facultative microorganisms
Inventor: James Copeland, INV
Kathy Myers, INV
Correspondence Address: FAY, SHARPE, FAGAN, MINNICH & McKEE, LLP, 7th Floor 1100 Superior Avenue, Cleveland, OH, 44114-2516, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20030138867	A1	20030724	US 20017739	20011108
Provisional				US 60-246872	20001108

Fulltext Word Count: 12721
?

Set	Items	Description
S1	35	'OXYRASE'
S2	0	S1 AND AZIDE?
S3	1	OXYRASE? AND AZIDE?
S4	263	OXYGENASE? AND AZIDE?
S5	66	S4/2000:2005
S6	197	S4 NOT S5
S7	9	S6 AND ANAEROB

WEST

Generate Collection | Print

L6: Entry 44 of 176 File: USPT Aug 4, 1998

DOCUMENT-IDENTIFIER: US 5789191 A
TITLE: Method of detecting and counting microorganisms

CLAIMS:

13. The method according to claim 1, wherein the selective medium is for testing enterococci and consists of 30 to 60 parts by weight of a customary base medium and one or more selectors selected from the group consisting of sodium citrate, sodium azide, thallium acetate and 2,3,5-triphenyltetrazole.

WEST

Generate Collection Print

L30: Entry 9 of 28 File: USPT Apr 6, 1999

DOCUMENT-IDENTIFIER: US 5891499 A
TITLE: Composition for eliminating unsanitary residues from food products and method for using the same

Detailed Description Text (23):
The used culture media were triptosium agar for the total count, the McConvey's soil for E. Coli and coliforms, and the Escherichia azide agar soil for the Streptococci D.

that produced optimal specific activity (125 to 175 nmol of C2H2
reduced/min per mg of total protein). The apparent Michaelis constants
(Km)for the magnesium adenosine triphosphate complex, reducible substrates
azide , acetylene, and N2 and the nonphysiological electron donor
hydrosulfite (S2O42-) were determined to be 0.7, 0.7, 0.2, 0.06, and 0.03
MM, respectively. These apparent Km values are in reasonable agreement with
those reported for the nitrogenases of Azotobacter vinelandii and
Klebsiella pneumoniae. Either a total lack of cooperativity between binding
sites or a single binding site for reducible substrates is indicated by
analysis of Hill plots. Hill plot slopes of approximately 1.7 suggest that
multiple binding sites exist for both ATP and S2O42-.
Tags: Support, U.S. Gov't, Non-P.H.S.
Descriptors: *Bacillus--enzymology--EN; *Nitrogenase--metabolism--ME; Acetylene--metabolism--ME; Adenosine Triphosphate--metabolism--ME; Anaerobiosis ; Azides --metabolism--ME; Azotobacter--enzymology--EN; Binding Sites; Cell-Free System; Kinetics; Klebsiella pneumoniae --enzymology--EN; Nitrogen--metabolism--ME; Species Specificity; Sulfites --metabolism--ME
CAS Registry No.: 0 (Azides); 0 (Sulfites); 56-65-5 (Adenosine Triphosphate); 74-86-2 (Acetylene); 7727-37-9 (Nitrogen)
Enzyme No.: EC 1.18.6.1 (Nitrogenase)
Record Date Created: 19760706

6/9/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12190987 BIOSIS NO.: 199900485836
Recovery of Escherichia coli Biotype I and Enterococcus spp. during refrigerated storage of beef carcasses inoculated with a fecal slurry.
AUTHOR: Calicioglu M; Buege D R; Ingham S C; Luchansky J B(a)
AUTHOR ADDRESS: (a)Department of Food Science, and Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, Madison, WI, 53706**USA
JOURNAL: Journal of Food Protection 62 (8):p944-947 Aug., 1999
ISSN: 0362-028X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Three beef front quarters/carcasses were inoculated with a slurry of cattle manure. During storage at 4degreeC, two sponge samples from each of three sites (i.e., 100 cm2 from each of two fat surfaces and 100 cm2 from a lean surface) were taken from each of the three carcasses on days 0, 1, 3, 7, and 10 after inoculation. The initial numbers of Escherichia coli averaged 2.0 log10 CFU/cm2 (1.21 to 2.47 log10 CFU/cm2) using the Petrifilm method and 2.09 log10 most probable number (MPN)/cm2 (0.88 to 2.96 log10 MPN/cm2) using the MPN method. The initial numbers of enterococci averaged 3.34 log10 CFU/cm2 (3.07 to 3.79 log10 CFU/cm2) using kanamycin esculin azide agar. In general, an appreciable reduction in the numbers of E. coli occurred during the first 24 h of storage; for the Petrifilm method an average reduction of 1.37 log10 CFU/cm2 (0.69 to 1.71 log10 CFU/cm2) was observed, and for the MPN method an average reduction of 1.52 log10 MPN/cm2 (0.47 to 2.08 log10 MPN/cm2) was observed. E. coli were not detected (<-0.12 log10 CFU/cm2) using Petrifilm on day 7 of the storage period on two (initial counts of 1.21 and 2.29 log10 CFU/cm2) of the three carcasses. However, viable E. coli cells were recovered from these two carcasses after a 24-h enrichment at 37degreeC in EC broth. Viable E. coli cells were detected at levels of -0.10 log10 CFU/cm2 on the third carcass (initial count of 2.47 log10 CFU/cm2) after 7 days at 4degreeC. No significant difference in recovery of viable cells was observed between the MPN and Petrifilm methods on days 0, 1, and 3 ($P > 0.05$). However, viable E. coli cells were recovered from all three carcasses by the MPN method on day 7 at an average of -0.29 log10 MPN/cm2 (-0.6 to -0.1 log10 MPN/cm2). On day 10, viable cells were recovered by the MPN method from two of the three carcasses at -0.63 and -0.48 log10 MPN/cm2 but were not recovered from the remaining carcass (<-0.8 log10 MPN/cm2).Similar to E. coli, the greatest reduction (average of 1.26 log10 CFU/cm2, range = 1.06 to 1.45 log10 CFU/cm2) in the numbers

of enterococci occurred during the first 24 h of storage. Because of
higher initial numbers and a slightly slower rate of decrease, the
numbers of Enterococcus spp. were significantly higher ($P < 0.017$) than
the numbers of E. coli Biotype I after 3, 7, and 10 days of storage.
These results suggest that enterococci may be useful as an indicator of
fecal contamination of beef carcasses.

DESCRIPTORS:
MAJOR CONCEPTS: Foods; Methods and Techniques
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Gram-Positive
Cocci--Eubacteria, Bacteria, Microorganisms
ORGANISMS: Enterococcus spp. (Gram-Positive Cocci)--food contaminant;
Escherichia coli (Enterobacteriaceae)--biotype I, food contaminant
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
METHODS & EQUIPMENT: most probable number method--food contaminant
detection method; refrigerated storage--food storage method;
Petrifilm method--food contaminant detection method
MISCELLANEOUS TERMS: beef carcasses--fecal contamination, meat
CONCEPT CODES:
39008 Food and Industrial Microbiology-General and Miscellaneous
13502 Food Technology-General; Methods
23001 Temperature: Its Measurement, Effects and Regulation-General
Measurement and Methods
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)
07700 Gram-Positive Cocci (1992-)

6/9/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12333320 BIOSIS NO.: 200000086822
Mechanism of anti- and pro-oxidant effect of azide on pentachlorophenol
metabolite-induced toxicity.
AUTHOR: Zhu Ben-Zhan(a); Levy Smadar(a); Chevion Mordechai(a)
AUTHOR ADDRESS: (a)Department of Cellular Biochemistry, Hebrew
University-Hadassah Medical School, Jerusalem, 91120**Israel
JOURNAL: Free Radical Biology & Medicine 27 (SUPPL. 1):pS127 1999
CONFERENCE/MEETING: 6th Annual Meeting of the Oxygen Society New Orleans,
Louisiana, USA November 18-22, 1999
SPONSOR: The Oxygen Society
ISSN: 0891-5849
RECORD TYPE: Citation
LANGUAGE: English
REGISTRY NUMBERS: 66-71-7: 1 10-PHENANTHROLINE; 14343-69-2: AZIDE ; 7440-50-8: COPPER; 70-51-9: DESFERRIOXAMINE; 1198-55-6:
TETRACHLOROCATECHOL
DESCRIPTORS:
MAJOR CONCEPTS: Metabolism; Toxicology
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia coli (Enterobacteriaceae)--model system
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: 1,10-phenanthroline; azide --preservative,
toxin; copper; desferrioxamine; tetrachlorocatechol--preservative,
toxin
METHODS & EQUIPMENT: ESR--analytical method, spectroscopic techniques--CB
, spectroscopic techniques--CT; UV/visible studies--radiobiology
method
MISCELLANEOUS TERMS: cytotoxicity; wood; Meeting Abstract
CONCEPT CODES:
22501 Toxicology-General; Methods and Experimental
06502 Radiation-General
31000 Physiology and Biochemistry of Bacteria
00520 General Biology-Symposia, Transactions and Proceedings of
Conferences, Congresses, Review Annuals
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/21 (Item 21 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11827656 BIOSIS NO.: 199900073765
The inherent genotoxic potency of food mutagens and other heterocyclic and
carbocyclic aromatic amines and corresponding azides .
AUTHOR: Wild D(a); Kerdar R S
AUTHOR ADDRESS: (a)Fed. Cent. Meat Res., Inst. Microbiol. Toxicol.,
E.-C.-Baumann-Str. 20, D-95326 Kulmbach**Germany
JOURNAL: Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung A 207 (
6):p427-433 1998

ISSN: 1431-4630
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Relationships between the chemical structure of aromatic amines
(including heterocyclic food mutagens) and genotoxic potency were
originally established on the basis of Salmonella mutagenicity data.
These relationships are reviewed. We report here that also quite
different genotoxic effects, namely the binding to deoxyguanosine-3'-phosphate (dGp), hypoxanthine phosphoribosyl-transferase (HPRT) mutations, and sister chromatid
exchange in Chinese hamster cells follow essentially the same
structure-activity relationships. The heterocyclic amines of the
aminoimidazoquinoline, aminoimidazoquinoxaline and aminoimidazopyridine
types unite a number of structural characteristics which endow these
compounds, or rather their reactive species, presumed to be nitrenium
ions, with an extremely high inherent genotoxic potency. This conclusion
is supported by experimental work and by calculations of electronic
properties of these compounds and their nitrenium ions.

REGISTRY NUMBERS: 14343-69-2D: AZIDES
DESCRIPTORS:
MAJOR CONCEPTS: Foods; Molecular Genetics (Biochemistry and Molecular
Biophysics); Toxicology
BIOSYSTEMATIC NAMES: Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata
, Animalia; Enterobacteriaceae-- Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Salmonella typhimurium (Enterobacteriaceae); V79 cell line
(Cricetidae)--Chinese hamster fibroblasts
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Animals; Bacteria; Chordates;
Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman
Vertebrates; Rodents; Vertebrates
CHEMICALS & BIOCHEMICALS: azides --food residue, quantitative
structure-activity relationships, genotoxicity; carbocyclic aromatic
amines--food residue, genotoxicity, quantitative structure-activity
relationships; heterocyclic aromatic amines--food residue, quantitative structure-activity relationships, genotoxicity
METHODS & EQUIPMENT: hypoxanthine ribosyltransferase assay--analytical
method; sister chromatid exchange test--assessment method; Ames test
--assessment method
MISCELLANEOUS TERMS: food products--cooked
CONCEPT CODES:
13502 Food Technology-General; Methods
02506 Cytology and Cytochemistry-Animal
03502 Genetics and Cytogenetics-General
10060 Biochemical Studies-General
10502 Biophysics-General Biophysical Studies
22501 Toxicology-General; Methods and Experimental
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)
86310 Cricetidae

6/9/16 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11940555 BIOSIS NO.: 199900186664
Active efflux and diffusion are involved in transport of Pseudomonas·
aeruginosa cell-to-cell signals.
AUTHOR: Pearson James P; van Delden Christian; Iglewski Barbara H(a)
AUTHOR ADDRESS: (a)Department of Microbiology and Immunology, University of
Rochester, 601 Elmwood Ave., Rochester,**USA
JOURNAL: Journal of Bacteriology 181 (4):p1203-1210 Feb., 1999
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Many gram-negative bacteria communicate by N-acyl homoserine
lactone signals called autoinducers (AIs). In Pseudomonas aeruginosa,
cell-to-cell signaling controls expression of extracellular virulence
factors, the type II secretion apparatus, a stationary-phase sigma factor
(sigmas), and biofilm differentiation. The fact that a similar signal,
N-(3-oxohexanoyl) homoserine lactone, freely diffuses through Vibrio
fischeri and Escherichia coli cells has led to the assumption that all
AIs are freely diffusible. In this work, transport of the two P.
aeruginosa AIs, N-(3-oxododecanoyl) homoserine lactone (3OC12-HSL)
(formerly called PAI-1) and N-butyryl homoserine lactone (C4-HSL)
(formerly called PAI-2), was studied by using tritium-labeled signals.
When (3H)C4-HSL was added to cell suspensions of P. aeruginosa, the
cellular concentration reached a steady state in less than 30 s and was

nearly equal to the external concentration, as expected for a freely
diffusible compound. In contrast, (3H)3OC12-HSL required about 5 min to
reach a steady state, and the cellular concentration was 3 times higher
than the external level. Addition of inhibitors of the cytoplasmic
membrane proton gradient, such as azide , led to a strong increase in
cellular accumulation of (3H)3OC12-HSL, suggesting the involvement of
active efflux. A defined mutant lacking the mexA-mexB-oprM-encoded
active-efflux pump accumulated (3H)3OC12-HSL to levels similar to those
in the azide -treated wild-type cells. Efflux experiments confirmed
these observations. Our results show that in contrast to the case for
C4-HSL, P. aeruginosa cells are not freely permeable to 3OC12-HSL.
Instead, the mexA-mexB-oprM-encoded efflux pump is involved in active
efflux of 3OC12-HSL. Apparently the length and/or degree of substitution
of the N-acyl side chain determines whether an AI is freely diffusible or
is subject to active efflux by P. aeruginosa.

REGISTRY NUMBERS: 672-15-1QD: HOMOSERINE; 1927-25-9QD: HOMOSERINE
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Chemical
Coordination and Homeostasis; Infection
BIOSYSTEMATIC NAMES: Bacteria--Microorganisms; Enterobacteriaceae--
Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria,
Microorganisms; Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci,
Eubacteria, Bacteria, Microorganisms
ORGANISMS: gram-negative bacteria (Bacteria); Escherichia coli
(Enterobacteriaceae)--pathogen; Pseudomonas aeruginosa
(Pseudomonadaceae)--pathogen
ORGANISMS: PARTS ETC: secretion apparatus
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: sigma factors; N-acyl homoserine lactone
MISCELLANEOUS TERMS: cell-to-cell signalling; efflux pumps; virulence
factors
CONCEPT CODES:
31000 Physiology and Biochemistry of Bacteria
10060 Biochemical Studies-General
12002 Physiology, General and Miscellaneous-General
12502 Pathology, General and Miscellaneous-General
36002 Medical and Clinical Microbiology-Bacteriology
13002 Metabolism-General Metabolism; Metabolic Pathways
30500 Morphology and Cytology of Bacteria
BIOSYSTEMATIC CODES:
05000 Bacteria-General Unspecified (1992-)
06508 Pseudomonadaceae (1992-)
06702 Enterobacteriaceae (1992-)

6/9/14 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11948709 BIOSIS NO.: 199900194818
Role of the lateral channel in catalase HPII of Escherichia coli.
AUTHOR: Sevinc M Serdal; Mate Maria J; Switala Jack; Fita Ignacio; Loewen
Peter C(a)
AUTHOR ADDRESS: (a)Department of Microbiology, University of Manitoba,
Winnipeg, MB, R3T 2N2**Canada
JOURNAL: Protein Science 8 (3):p490-498 March, 1999
ISSN: 0961-8368
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The heme-containing catalase HPII of Escherichia coli consists of
a homotetramer in which each subunit contains a core region with the
highly conserved catalase tertiary structure, to which are appended N-
and C-terminal extensions making it the largest known catalase. HPII does
not bind NADPH, a cofactor often found in catalases. In HPII, residues
585-590 of the C-terminal extension protrude into the pocket
corresponding to the NADPH binding site in the bovine liver catalase.
Despite this difference, residues that define the NADPH pocket in the
bovine enzyme appear to be well preserved in HPII. Only two residues that
interact ionically with NADPH in the bovine enzyme (Asp212 and His304)
differ in HPII (Glu270 and Glu362), but their mutation to the bovine
sequence did not promote nucleotide binding. The active-site heme groups
are deeply buried inside the molecular structure requiring the movement
of substrate and products through long channels. One potential channel is
about 30 ANG in length, approaches the heme active site laterally, and is
structurally related to the branched channel associated with the NADPH

binding pocket in catalases that bind the dinucleotide. In HPII, the
upper branch of this channel is interrupted by the presence of Arg260
ionically bound to Glu270. When Arg260 is replaced by alanine, there is a
threefold increase in the catalytic activity of the enzyme. Inhibitors of
HPII, including azide , cyanide, various sulfhydryl reagents, and
alkylhydroxylamine derivatives, are effective at lower concentration on
the Ala260 mutant enzyme compared to the wild-type enzyme. The crystal
structure of the Ala260 mutant variant of HPII, determined at 2.3 ANG
resolution, revealed a number of local structural changes resulting in
the opening of a second branch in the lateral channel, which appears to
be used by inhibitors for access to the active site, either as an inlet
channel for substrate or an exhaust channel for reaction products.

REGISTRY NUMBERS: 9001-05-2: CATALASE; 14875-96-8: HEME; 9001-05-2: EC
1.11.1.6; 58-68-4: NADH
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology (Biochemistry and Molecular Biophysics)
BIOSYSTEMATIC NAMES: Animalia; Bacteria--Microorganisms;
Enterobacteriaceae-- Facultatively Anaerobic Gram-Negative Rods,
Eubacteria, Bacteria, Microorganisms; Fungi--Plantae
ORGANISMS: animals (Animalia); bacteria (Bacteria); yeasts (Fungi);
Escherichia coli (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Animals; Bacteria; Eubacteria;
Fungi; Microorganisms; Nonvascular Plants; Plants
CHEMICALS & BIOCHEMICALS: catalase HPII {EC 1.11.1.6}--analysis,
heme-containing enzyme, lateral channel roles, molecular
characteristics; heme; oligonucleotides; reaction products--analysis
; solvents; NADH
METHODS & EQUIPMENT: enzyme purification--Isolation/Purification
Techniques--CB, purification method; mutagenesis--molecular genetic
method, molecular genetics/genetic engineering,
oligonucleotide-directed; PCR-Mate synthesizer--Applied Biosystems,
equipment; X-ray crystallography--X-ray analysis, analytical method
CONCEPT CODES:
31000 Physiology and Biochemistry of Bacteria
10050 Biochemical Methods-General
10060 Biochemical Studies-General
10502 Biophysics-General Biophysical Studies
10506 Biophysics-Molecular Properties and Macromolecules
10802 Enzymes-General and Comparative Studies; Coenzymes
10806 Enzymes-Chemical and Physical
BIOSYSTEMATIC CODES:
05000 Bacteria-General Unspecified (1992-)
06702 Enterobacteriaceae (1992-)
15000 Fungi-Unspecified
33000 Animalia-Unspecified

6/9/15 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11941209 BIOSIS NO.: 199900187318
Conditional stability of the HemA protein (glutamyl-tRNA reductase)
regulates heme biosynthesis in Salmonella typhimurium.
AUTHOR: Wang Liying; Elliott Meenal; Elliott Thomas(a)
AUTHOR ADDRESS: (a)Department of Microbiology and Immunology, WVU Health
Sciences Center, Morgantown, WV, 26506-917**USA
JOURNAL: Journal of Bacteriology 181 (4):p1211-1219 Feb., 1999
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In many bacteria, including the enteric species Salmonella
typhimurium and Escherichia coli, heme is synthesized starting from
glutamate by a pathway in which the first committed step is catalyzed by
the hemA gene product, glutamyl-tRNA reductase (HemA). We have
demonstrated previously that when heme limitation is imposed on cultures
of S. typhimurium, HemA enzyme activity is increased 10- to 25-fold.
Western (immunoblot) analysis with monoclonal antibodies reactive with
HemA revealed that heme limitation results in a corresponding increase in
the abundance of the enzyme. Similar regulation was also observed for E.
coli. The near absence of regulation of hemA-lac operon fusions suggested
a posttranscriptional control. We report here the results of
pulse-labeling and immunoprecipitation studies of this regulation. The
principal mechanism that contributes to elevated HemA abundance is
protein stabilization. The half-life of HemA protein is simeq20 min in
unrestricted cells but increases to >300 min in heme-limited cells.

PCL XL error

Subsystem: IMAGE

Error: ExtraData

Operator: ReadImage

Position: 234

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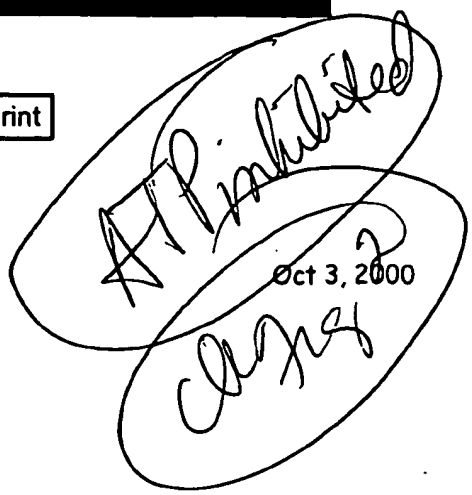

L19: Entry 4 of 11 File: USPT Oct 3, 2000

DOCUMENT-IDENTIFIER: US 6127167 A
TITLE: Method of controlling proliferation of aerobe

Abstract Text (1):
Disclosed herein is a method of controlling the proliferation of an aerobe, in which in the continuous culture of the aerobe by supplying a substrate and oxygen to a culture tank of the aerobe to cause the aerobe to decompose the substrate, the proliferation of the aerobe is inhibited while retaining the substrate-decomposing activity inherent in the aerobe. An oxidation-reduction substance which is reduced by electrons donated by an electron transport system of the aerobe and oxidized by oxygen supplied to the culture tank is caused to coexist with the aerobe in the culture tank.

Brief Summary Text (14):
In order to achieve the above object, in an aspect of the present invention, there is thus provided a method of controlling the proliferation of an aerobe, in which in the continuous culture of the aerobe by supplying a substrate and oxygen to a culture tank of the aerobe to cause the aerobe to decompose the substrate, the proliferation of the aerobe is inhibited while retaining the substrate-decomposing activity inherent in the aerobe, wherein an oxidation-reduction substance which is reduced by electrons donated by an electron transport system of the aerobe and oxidized by oxygen supplied to the culture tank is caused to coexist with the aerobe in the culture tank.

Detailed Description Text (12):
According to the method of the present invention, the oxidation-reduction substance coexists with the aerobe in the culture tank. Therefore, in the electron transport system, the electrons formed by the charge separation at the coupling site 13 or the coupling site 17 are donated to the oxidation-reduction substance. As a result, the concentration gradient of proton formed in the above-described manner becomes insufficient, and so the synthesis of ATP in the electron transport system is inhibited. ATP synthesized in the electron transport system accounts for about 70% of ATP formed in the whole aerobic respiration system. Therefore, if the synthesis of ATP in the electron transport system is inhibited, ATP is lacking, and so the proliferation is inhibited.

Detailed Description Text (15):
The oxygen used may be supplied in the form of a mixture with other one or more gases. Air is generally supplied as a gas containing oxygen. Preferable examples of the aerobe include aerobic bacteria, facultative anaerobic bacteria, actinomycete, yeast, mold and basidiomycete. These aerobes may be used either singly or in any combination thereof.

Detailed Description Text (30):

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L4: Entry 46 of 57 File: USPT Nov 8, 1983

DOCUMENT-IDENTIFIER: US 4414334 A
TITLE: Oxygen scavenging with enzymes

Detailed Description Text (42):
At the end of dialysis, the alcohol oxidase is present in the dialysis bag as a crystalline solid. The crystalline alcohol oxidase can be readily separated from the dialysis medium, such as by decanting the liquid in the dialysis bag from the solid crystals. The moist crystals can be further processed as desired for storage. For example, the crystal slurry can be frozen followed by lyophilization to form a dry powder, or can be dissolved in water or more preferably in a phosphate buffer. The alcohol oxidase can be stored frozen without significant loss of enzymatic activity. Stabilizer compounds known to stabilize enzyme solutions against denaturation and loss of enzymatic activity can be added, such as sucrose or glycerol, or 0.02 weight % sodium azide.

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United States Patent [19]

Hitzman

[11] **4,414,334**

[45] **Nov. 8, 1983**

[54] **OXYGEN SCAVENGING WITH ENZYMES**

[75] Inventor: **Donald O. Hitzman, Bartlesville, Okla.**

[73] Assignee: **Phillips Petroleum Company, Bartlesville, Okla.**

[21] Appl. No.: **291,146**

[22] Filed: **Aug. 7, 1981**

[51] **Int. Cl.**[3] **C12N 9/04**; C10G 32/00; C12R 1/84

[52] **U.S. Cl.** **435/262**; 426/7; 426/12; 435/190; 435/281; 435/938

[58] **Field of Search** 435/190, 262, 264, 247, 435/938, 281; 426/7, 10, 8, 34, 12

[56] **References Cited**

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Primary Examiner—David M. Naff

[57] **ABSTRACT**

Removal of ambient oxygen from aqueous liquids is effectively catalyzed by enzymatic deoxygenation systems comprising alcohol oxidase in the presence of alcohol optionally with catalase. Suitable deoxygenation systems described can be used to alleviate corrosion and oxidative degradation in areas such as oil field fluids, circulating water systems, water storage tanks, alcoholic beverages and foodstuffs. As desired, the enzymatic systems can be immobilized on supports or used in solution.

17 Claims, 3 Drawing Figures

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L2: Entry 15 of 18 File: USPT Feb 7, 1984

DOCUMENT-IDENTIFIER: US 4430427 A
TITLE: Red absorbing combination of alcohol oxidase and an azide compound

CLAIMS:

1. A composition of matter comprising active alcohol oxidase; and

an azide compound selected from the group of compounds having the formula R"(N.sub.3).sub.x wherein R" is a metal atom, a hydrogen atom, or the ammonium radical, and N.sub.3 is the moiety N.dbd.N.dbd.N,

the azide compound being present in an amount effective to form a red absorbing combination with active alcohol oxidase.

3. A composition as in claim 1 wherein:

the azide is present in an amount in the range of about 1 mole azide/mole alcohol oxidase to about 5.0.times.10.sup.7 moles azide/mole alcohol oxidase.

4. A composition as in claim 1 wherein:

the azide is present in an amount in the range of about 8 moles azide/mole alcohol oxidase to about 5.0.times.10.sup.7 moles azide/mole alcohol oxidase.

5. A composition as in claim 1 wherein:

the azide is present in an amount in the range of about 10 moles azide/mole alcohol oxidase to about 2000 moles azide/mole alcohol oxidase.

6. A composition as in claim 1 wherein:

the alcohol oxidase is isolated from genus Pichia; and

the azide is present in an amount in the range of about 0.07 mg azide/gram alcohol oxidase to about 80,000 mg azide/gram alcohol oxidase.

7. A composition as in claim 1 wherein:

the alcohol oxidase is isolated from genus Pichia; and

the azide is present in an amount in the range of about 0.1 mg azide/gram alcohol oxidase to about 80,000 mg azide/gram alcohol oxidase.

8. A composition as in claim 1 wherein:

the alcohol oxidase is isolated from genus Pichia; and

the azide is present in an amount in the range of about 0.7 mg azide/gram alcohol oxidase to about 135 mg azide/gram alcohol oxidase.

12. A method of purifying an active alcohol oxidase comprising:

adding an azide compound selected from the group of compounds having the formula R"(N.sub.3).sub.x wherein R" is a metal atom, a hydrogen atom, or the ammonium radical, and N.sub.3 is the moiety N.dbd.N.dbd.N to a preparation comprising the active alcohol oxidase in an amount effective for producing a red absorbing complex; and separating the red absorbing complex from the preparation.

13. A method as in claim 12 wherein adding an azide comprises:

adding an amount of sodium azide in the range of about 1 mole azide/mole alcohol oxidase to about 5.0.times.10.sup.7 moles azide/mole alcohol oxidase.

14. A method as in claim 12 wherein adding an azide comprises:

adding an amount of sodium azide in the range of about 8 moles azide/mole alcohol oxidase to about 5.0.times.10.sup.7 moles azide/mole alcohol oxidase.

15. A method as in claim 12 wherein adding an azide comprises:

adding an amount of sodium azide in the range of about 10 moles azide/mole alcohol oxidase to about 2000 moles azide/mole alcohol oxidase.

16. A method as in claim 12 wherein adding an azide comprises:

adding an amount of sodium azide in the range of about 0.07 mg azide/gram alcohol oxidase to about 80,000 mg azide/gram alcohol oxidase; and wherein

the alcohol oxidase is isolated from genus Pichia.

17. A method as in claim 12 wherein adding an azide comprises:

adding an amount of sodium azide in the range of about 0.1 mg azide/gram alcohol oxidase to about 80,000 mg azide/gram alcohol oxidase; and wherein

the alcohol oxidase is isolated from genus Pichia.

18. A method as in claim 12 wherein adding an azide comprises:

adding an amount of sodium azide in the range of about 0.7 mg azide/gram alcohol oxidase to about 135 mg azide/gram alcohol oxidase; and wherein

the alcohol oxidase is isolated from genus Pichia.

28. A method of determining the presence of active alcohol oxidase comprising:

observing the color of a composition of matter comprising an alcohol oxidase and an azide compound selected from the group of compounds having the formula R"(N.sub.3).sub.x wherein R" is a metal atom, a hydrogen atom, or the ammonium radical, and N.sub.3 is the moiety

N.dbd.N.dbd.N

the azide compound being present in an amount effective to form a red absorbing combination with active alcohol oxidase.

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United States Patent [19]

Hopkins

[11] **4,430,427**

[45] **Feb. 7, 1984**

[54] **RED ABSORBING COMBINATION OF ALCOHOL OXIDASE AND AN AZIDE COMPOUND**

[75] Inventor: **Thomas R. Hopkins, Bartlesville, Okla.**

[73] Assignee: **Phillips Petroleum Company, Bartlesville, Okla.**

[21] Appl. No.: **203,923**

[22] Filed: **Nov. 4, 1980**

[51] **Int. Cl.**[3] **C12Q 1/26**; C12N 9/02; C12N 9/04; C12N 9/96

[52] **U.S. Cl.** **435/25**; 435/188; 435/189; 435/190; 435/814; 435/815; 435/816

[58] **Field of Search** 435/25, 4, 190, 188, 435/189, 174, 177, 180, 814, 815, 816

[56] **References Cited**

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Primary Examiner—David M. Nafe

[57] **ABSTRACT**

Combining active alcohol oxidase with sufficient amount of an azide compound has been found to form a red absorbing combination. Formation of the red absorbing combination enables determining the presence of active alcohol oxidase by adding the azide compound to a preparation and observing the resultant color. Additionally, alcohol oxidase may be purified by adding the azide compound to a preparation containing active alcohol oxidase in an amount effective to produce a red absorbing complex and separating the red absorbing complex from the preparation.

28 Claims, No Drawings

DOCUMENT-IDENTIFIER: US 4894339 A
TITLE: Immobilized enzyme membrane for a semiconductor sensor

CLAIMS:

11. A membrane as set forth in claim 1 wherein said polyvinyl pyrrolidone having a high molecular weight is 2-20 weight %; and said 2, 5-bis (4'-azide-2'-sulfobenzal) cyclopentanone sodium salt is 0.5-1.5 weight %; and said enzyme comprises glucose oxidase of 5.0-7.5 weight %; said membrane further comprising bovine serum albumin of 5-10 weight %.

22. A method for producing a membrane as set forth in claim 12 wherein said polyvinyl pyrrolidone having a high molecular weight is 2-20 weight %; and said 2, 5-bis (4'-azide-2'-sulfobenzal) cyclopentanone sodium salt is 0.5-1.5 weight %; and said enzyme comprises glucose oxidase of 5.0-7.5 weight %; said aqueous solution further comprising bovine serum albumin of 5-10 weight %.

48. A sensor containing an enzyme as set forth in claim 28 wherein said polyvinyl pyrrolidone having a high molecular weight is 2-20 weight %; and said 2,5-bis (4'-azide-2'-sulfobenzal) cyclopentanone sodium salt is 0.5-1.5 weight %; and said enzyme comprises glucose oxidase of 5.0-7.5 weight %; said membrane containing a first immobilized enzyme further comprising bovine serum albumin of 5-10 weight %.

49. A sensor containing an enzyme as set forth in claim 29 wherein said polyvinyl pyrrolidone having a high molecular weight is 2-20 weight %; and said 2, 5-bis (4'-azide-2'-sulfobenzal) cyclopentanone sodium salt is 0.5-1.5 weight %; and said enzyme comprises glucose oxidase of 5.0-7.5 weight %; said membrane containing a second immobilized enzyme further comprising bovine serum albumin of 5-10 weight %.

United States Patent [19]

Hanazato et al.

[11] **Patent Number: 4,894,339**

[45] **Date of Patent: Jan. 16, 1990**

[54] **IMMOBILIZED ENZYME MEMBRANE FOR A SEMICONDUCTOR SENSOR**

[75] Inventors: **Yoshio Hanazato; Satoru Shiono; Mamiko Nakako; Satoshi Yamada**, all of Hon, Japan

[73] Assignee: **Seitaikinouriyou Kagakuhin Sinseizogijutsu Kenkyu Kumiai**, Japan

[21] Appl. No.: **942,797**

[22] Filed: **Dec. 17, 1986**

[30] **Foreign Application Priority Data**

Dec. 18, 1985	[JP]	Japan	60-283056
Jan. 27, 1986	[JP]	Japan	61-13900
Jan. 27, 1986	[JP]	Japan	61-13902

[51] **Int. Cl.**[4] **C12N 11/04**; C12N 11/08; C12M 1/40; G01N 27/26

[52] **U.S. Cl.** **435/182**; 204/403; 435/180; 435/288; 435/817

[58] **Field of Search** 435/174, 177, 180, 182, 435/817, 288; 204/403

[56] **References Cited**

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Primary Examiner—David M. Naff
Attorney, Agent, or Firm—Leydig, Voit & Mayer

[57] **ABSTRACT**

A membrane containing an immobilized enzyme for a semiconductor sensor is prepared containing a water soluble photosensitive resin including a high molecular weight polyvinyl pyrrolidone crosslinked to 2, 5-bis (4'-azide-2'-sulfobenzal) cyclopentanone sodium salt, and an enzyme. Glutaraldehyde and bovine serum albumin, polyamino acid or polyamino amino acid copolymer may also be present to provide chemical crosslinking. The enzyme may be glucose oxidase, urease or lipase. The membrane can be directly formed on ion-sensitive protions of a pH-ion sensitive field effect transistor to form a semiconductor sensor by coating an aqueous solution of the resin and enzyme on the ion-sensitive portion, drying and irradiating with light such as ultraviolet light to provide photo crosslinking.

56 Claims, 9 Drawing Sheets

DOCUMENT-IDENTIFIER: US 4810633 A
TITLE: Enzymatic ethanol test

CLAIMS:

6. A method for the manufacture of a dry solid state reagent strip for determining ethanol in body fluids in concentrations as low as 100 mg/dL anc capable of providing an anlytical, colorimetric response in less than 5 minutes, said method comprising the steps of:

(a) preparing a first mixture of a single component chromogenic indicator and an organic solvent;

(b) preparing an aqueous second mixture of a peroxidatively active substance, any remaining components of the chromogenic indicator system, from about 0.5 to about 5 mM alkali metal azide and from about 20 to about 200 IU per milliliter alcohol oxidase;

(c) incorporating the carrier matrix with one of the first or second mixtures and drying; and

(d) incorporating the carrier with the other of the first or second mixtures and drying.

United States Patent [19]

Bauer et al.

[11] **Patent Number: 4,810,633**

[45] **Date of Patent: Mar. 7, 1989**

[54] **ENZYMATIC ETHANOL TEST**

[75] Inventors: **Robert Bauer, Bristol; Thomas A. Magers, South Bend, both of Ind.**

[73] Assignee: **Miles Inc., Elkhart, Ind.**

[21] Appl. No.: **616,732**

[22] Filed: **Jun. 4, 1984**

[51] Int. Cl.[4] **C12Q 1/26**; C12Q 1/28; C12N 9/96

[52] U.S. Cl. **435/25**; 435/28; 435/805; 435/188; 427/2

[58] Field of Search 422/56, 57; 435/4, 25, 435/28, 188, 805, 810; 427/2

[56] **References Cited**

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Primary Examiner—Esther M. Kepplinger

Attorney, Agent, or Firm—Roger N. Coe

[57] **ABSTRACT**

The present invention provides a stable, convenient solid state test device for the determination of ethanol in an aqueous test sample, a method for its preparation and a method for its use. The use of carrier matrix incorporated with alcohol oxidase, a peroxidatively active substance and a suitable chromogenic indicator for the determination of ethanol in aqueous samples such as body fluid samples (e.g., serum, urine or saliva) is part of the invention. The invention provides a method of incorporating the enzyme alcohol oxidase and a peroxidatively active substance into a carrier matrix with a chromogenic indicator system capable of providing a detectable response to the presence of at least 100 mg/dL ethanol in less than about 5 minutes. The method involves either (a) the use of a specialized incorporation procedure or (b) the addition of an azide to the test device. Either method overcomes the "false positive" problem seen when solution assay reagents are incorporated at the increased concentrations necessary to provide a test device sensitive to the at least 100 mg/dL ethanol.

6 Claims, No Drawings

DOCUMENT-IDENTIFIER: US 4485016 A
TITLE: Enzymatic removal of aromatic hydroxy compounds and aromatic amines from waste waters

CLAIMS:

10. An enzyme reagent system consisting essentially of (a) peroxidase, (b) one of alcohol oxidase or glucose oxidase, and (c) from 0.01 to 0.05 weight percent of an azide compound of the formula MN.sub.3 wherein M is NH.sub.4.sup.+ or an alkali metal wherein the ratio of (a) to (b) is 10:1 to 1:10.

11. An enzyme reagent system according to claim 10 wherein (b) is alcohol oxidase and said azide compound (c) is sodium azide.

12. An enzyme reagent system according to claim 10 wherein (b) is glucose oxidase and said azide compound (c) is sodium azide.

United States Patent [19]

Hopkins

[11] **Patent Number: 4,485,016**

[45] **Date of Patent: Nov. 27, 1984**

[54] **ENZYMATIC REMOVAL OF AROMATIC HYDROXY COMPOUNDS AND AROMATIC AMINES FROM WASTE WATERS**

[75] Inventor: **Thomas R. Hopkins**, Bartlesville, Okla.

[73] Assignee: **Phillips Petroleum Company**, Bartlesville, Okla.

[21] Appl. No.: **595,142**

[22] Filed: **Mar. 30, 1984**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 494,489, May 13, 1983, abandoned.

[51] **Int. Cl.**[3] **C02F 1/74**

[52] **U.S. Cl.** **210/632**; 210/717; 210/721; 210/759; 210/909; 435/262

[58] **Field of Search** 210/632, 606, 909, 759, 210/763, 721, 724, 717; 435/14, 25, 28, 262, 264, 188

[56] **References Cited**

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Primary Examiner—Benoit Castel

[57] **ABSTRACT**

A process for the removal of at least one compound selected from the group consisting of an aromatic hydroxy compound or an aromatic amine having a water solubility of at least 0.01 mg/L from waste water containing the same, which comprises treating the water with a treating agent which consists essentially of peroxidase, at least one agent selected from the group consisting of alcohol oxidase and a straight chain C_1 to C_4 alcohol or glucose oxidase and glucose and an azide salt of the formula MN_3.

14 Claims, No Drawings

DOCUMENT-IDENTIFIER: US 4254220 A
** See image for Certificate of Correction **
TITLE: Composition for the kinetic determination of glucose

CLAIMS:

1. A composition adapted for the kinetic determination of glucose, said composition comprising:

(a) glucose-oxidase and peroxidase enzymes;

(b) a buffer system;

(c) a compound containing in the molecule an aromatic ring with at least a phenolic --OH;

(d) a compound containing a group bonded to a 1-phenyl-5-pyrazolinone;

(e) a substance for the inhibition of the glucose oxidase enzyme, said substance being selected from the group consisting of nitrates, selenites, sulphites, thiocyanates, and semicarbazide.

United States Patent [19]

Meiattini

[11] **4,254,220**

[45] **Mar. 3, 1981**

[54] **COMPOSITION FOR THE KINETIC DETERMINATION OF GLUCOSE**

[75] Inventor: **Franco Meiattini, Siena, Italy**

[73] Assignee: **Sclavo, S.p.A., Milan, Italy**

[21] Appl. No.: **82,174**

[22] Filed: **Oct. 5, 1979**

[30] **Foreign Application Priority Data**

Oct. 12, 1978 [IT] Italy 28665 A/78

[51] **Int. Cl.**³ **C12Q 1/54**

[52] **U.S. Cl.** **435/14; 435/28**

[58] **Field of Search** 435/14, 28

[56] **References Cited**

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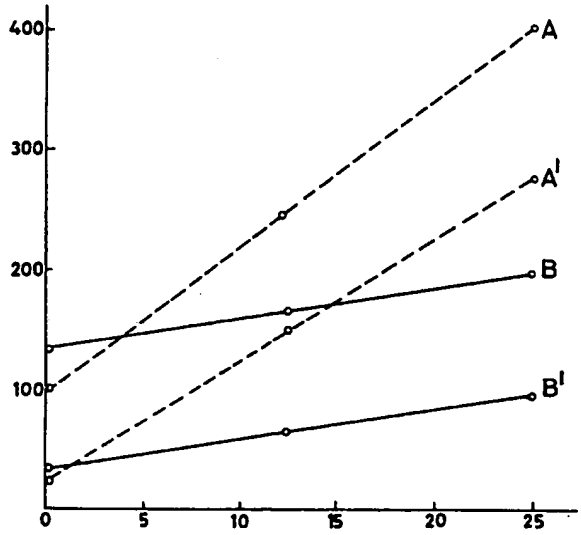
Primary Examiner—Thomas G. Wyse

Attorney, Agent, or Firm—Morgan, Finnegan, Pine, Foley & Lee

[57] **ABSTRACT**

A composition for the determination of glucose in the kinetic mode comprising the enzymes glucose-oxidase and peroxidase, a buffer system, a component containing an aromatic ring with a phenolic hydroxyl attached to said ring, a component containing a reactive group containing a structure derived from 1-phenyl-5-pyrazolinone and a substance which inhibits glucose-oxidase but not peroxidase.

5 Claims, 4 Drawing Figures



DOCUMENT-IDENTIFIER: US 6544797 B1
TITLE: Compositions and methods for inhibiting light-induced inactivation of biological reagents

Abstract Text (1):
Disclosed is a fluorescent conjugate comprising a biological reagent, a fluorescent molecule, and a means for impeding phototoxic degradation of the biological reagent. The impeding means can be a cross-linking substance having a long molecular distance, whereby the cross-linking substance links the fluorescent molecule and the biological reagent; a quencher of singlet oxygen; a quencher of a free radical; or a combination thereof. Also disclosed is a solution comprising a fluorescent conjugate of a biological reagent and a fluorescent molecule together with an oxygen depleting system.

Brief Summary Text (16):
Thus, disclosed is a fluorescent conjugate comprising a biological reagent, a fluorescent molecule, and a means for impeding phototoxic degradation of the biological reagent. The impeding means can comprise a cross-linking substance having a long molecular distance, whereby the cross-linking substance links the fluorescent molecule and the biological reagent; a protein; a quencher of singlet oxygen; a quencher of a free radical; a system for depleting oxygen; or a combination thereof.

Brief Summary Text (17):
The conjugate can comprise that the fluorescent molecule is directly linked to the biological reagent. The conjugate can further comprise a particle which comprises a fluorescent molecule; the particle can comprise a natural material or a synthetic material. For example, the particle can comprise a natural material which is alumina, silica, or, a liposome. The particle can further comprise an oxygen depleting system.

Brief Summary Text (18):
Disclosed is a solution comprising a fluorescent conjugate and an oxygen depleting system. For an embodiment of the fluorescent conjugate to be placed in a solution, the solution can comprise a means for impeding phototoxic degradation. The impeding means of the solution can comprise an antioxidant, a protein, an oxygen depleting system, or a combination thereof. The fluorescent conjugate of the solution can comprise a biological reagent, a fluorescent molecule, and a means for impeding phototoxic degradation of the biological reagent. The impeding means of the conjugate in the solution can comprise: a system to deplete oxygen; a cross-linking substance having a long molecular distance, whereby the cross-linking substance links the fluorescent molecule and the biological reagent; an antioxidant; a protein; or, a combination thereof.

Brief Summary Text (19):
Also disclosed is a fluorescent conjugate prepared by a process comprising steps of: providing a biological reagent; providing a fluorescent molecule; providing a heterofunctional linking reagent; chemically reacting the biological reagent, the fluorescent molecule, and the heterofunctional linking reagent, whereby the biological reagent becomes linked to the fluorescent molecule; and; associating an oxygen depleting system, a protein, an antioxidant, or a combination thereof, with the biological reagent or the fluorescent molecule prior to the reacting step; or, associating an oxygen depleting system, a protein, an antioxidant, or a combination thereof, with the linked biological reagent and fluorescent molecule following the reacting step. The heterofunctional linking reagent can be: a heterofunctional polyethylene glycol (PEG) derivative, a long chain form of succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), a long chain form of succinimidyl 3-(2-pyridyldithio)propionate (SPDP), or, a heterofunctional peptide derivative.

Brief Summary Text (20):

Disclosed is a method of use of an antioxidant, a protein, an oxygen depleting system, or a cross linking substance to impair the phototoxic degradation of a biological reagent in a fluorescent conjugate comprising the biological reagent and a fluorescent molecule, or to impair the phototoxic degradation of the material used to prepare a composition comprising a fluorescent molecule, the method occurring by combining an antioxidant, a protein, an oxygen depleting system, or a cross linking substance with the fluorescent conjugate, or with the material used to prepare a composition comprising a fluorescent molecule.

Brief Summary Text (35):
Reactive species--Examples of reactive species are singlet oxygen, free radicals and excited triplet states.

Brief Summary Text (38):
For the first time in the art, the mechanisms that lead to the degradation of biological reagents in fluorescent conjugates have been identified. It has been found that ambient white light leads to the generation of singlet oxygen or radicals which act on biological reagents. The studies that led to this finding are disclosed, as are compositions and methods to modulate this degradation. Pursuant to the present invention, the effectiveness of fluorescent conjugates for use in assays is enhanced.

Brief Summary Text (39):
Although the degradation of biological reagents in fluorescent systems had been noted, the causative mechanism was unknown. Surprisingly, it was found that biological reagents such as antibodies, peptides and ligand analogues, as well as reagents used to synthesize the fluorescent conjugates, are degraded by a mechanism that is induced upon exposure of these materials to routine ambient conditions. The ambient conditions can constitute mere exposure to white light and oxygen.

Brief Summary Text (42):
The inactivation of antibody binding activity in immunoassays or inactivation of nucleic acid hybridization assays by singlet oxygen or radicals has not been previously described. Previously, only the inactivation of lysozyme and the oxidation of amino acids have been described (Singlet Oxygen, 1985, Vol. IV, p. 91-143, ed. A. A. Frimer, CRC Press, Boca Raton, Fla.; Int. J. Radiat. Biol. (1986) 50:41-45); however, these descriptions are related to fluorescent dyes that are not conjugated to proteins, peptides, ligand analogues and nucleic acids; unlike the present invention, these dyes and biological reagents were freely soluble in aqueous solutions.

Brief Summary Text (43):
Furthermore, it has been noted that phthalocyanines can also initiate phototoxicity by an electron transfer mechanism (Type 1) in which the fluorescent dye, in an excited triplet state, can undergo reactions with neighboring molecules by an electron or hydrogen transfer process. The production of singlet oxygen (Type II) by the excitation of fluorescent dyes has been utilized for photodynamic therapy (Polym. Adv. Technol. (1995) 6:118-130; Int. J. Radiat. Biol. (1995) 67:85-91; Ciba Foundation Symposium (1989) 146:17-26) and in immunoassays, whereby singlet oxygen generation by particles comprised of silicon phthalocyanine has been used to generate a delayed luminescence. The delayed luminescence is proportional to the analyte concentration (Clin. Chem. 42:1518-1526 (1996)).

Brief Summary Text (44):
The teachings herein show that light (ambient or focused) decreased the effectiveness in binding reactions of antibodies, peptide and ligand analogue fluorescent conjugates. The mechanism for the decrease in effectiveness is believed to be related to the generation of reactive species, such as singlet oxygen or radicals, by the fluorescent molecules. The inactivation of biological reagents in fluorescent conjugates makes them unreliable for use in assays. The inactivation of antibody binding in

immunoassays or of nucleic acid hybridization in nucleic acid assays is generally manifest by a decrease in the slope of a dose response curve of the assay.

Brief Summary Text (46):
The presumed mode of degradation is type I and/or type II mechanisms of phototoxicity. In the case of type I mechanisms, the triplet sensitizer, that is, the triplet state of a fluorescent dye, reacts with nearby molecules through an electron or hydrogen transfer process. Free radicals are thus formed; namely the semireduced sensitizer and the semioxidized substrate. The semireduced sensitizer can react with oxygen to form a superoxide radical anion, which can further react with other molecules. The semioxidized sensitizer can become reduced by another molecule to form a semioxidized substrate, which can further react.

Brief Summary Text (47):
In the case of type II mechanisms, the triplet sensitizer can react with ground state oxygen to form singlet oxygen which can further react with other molecules, such as biomolecules and reagents to give oxidized products. (See, e.g., Int. J. Radiat. Biol. (1995) 67:85-91).

Brief Summary Text (48):
As disclosed herein, biological reagents associated with fluorescent dyes become inactivated or ineffective through the triplet state of the dye. The generation of triplet states of fluorescent dyes can result in the formation of a variety of radical species, including but not limited to singlet oxygen, superoxide radicals, hydroxyl radicals and organic radicals. These radical species have been known to cause damage to biological cells and proteins. Accordingly, these reactive triplet state species react with the surface of the biological reagent. Consequently, there will be a decreased effectiveness of an antibody for binding to analyte, or for a peptide or ligand analogue for binding to antibody. The inventive teachings set forth compositions which slow or prevent the light-induced destruction of biological reagents.

Brief Summary Text (51):
Accordingly, it has now been found that inactivation of biological reagents conjugated to fluorescent molecules or fluorescent particles can be decreased or prevented by use of radical scavengers termed quenchers, oxygen depleting systems in solutions that contain the fluorescent biological reagent conjugates, chemical linkages in conjugates to minimize the effects of radicals, or combinations thereof.

Brief Summary Text (52):
Another embodiment of this invention comprises a system which consumes dissolved oxygen. For example, a system of this embodiment comprises catalase, glucose oxidase, and glucose, as discussed in greater detail herein.

Brief Summary Text (53):
In a preferred embodiment, solutions containing chemically oxidizable compounds that are associated with or conjugated to fluorescent molecules, also contain singlet oxygen and/or free radical quenchers to minimize or prevent oxidation of the chemical species. Oxidizable compounds include those that are capable of being converted from one oxidation state to a higher one. In general, most oxidations involve a gain of oxygen and/or a loss of hydrogen.

Brief Summary Text (54):
In another preferred embodiment, solutions containing biological reagents that are associated with or conjugated to fluorescent molecules, also contain singlet oxygen and/or free radical quenchers to minimize or prevent inactivation of the biological reagent. Biological reagents include, peptides and proteins containing oxidizable amino acids or pendant molecules, and nucleic acids.

Brief Summary Text (55):
In another preferred embodiment, solutions containing antibodies that are associated with or conjugated to fluorescent molecules or particles also contain singlet oxygen and/or free radical quenchers to minimize or prevent the ability of the antibody to bind its antigen. Antibodies include, polyclorial and monoclonal antibodies and binding fragments generated by recombinant protein synthesis or other techniques.

Brief Summary Text (56):
In yet another preferred embodiment, solutions containing nucleic acids that are associated with or conjugated to fluorescent molecules, also contain singlet oxygen and/or free radical quenchers to minimize or prevent the inactivation or slow the rate of hybridization of the nucleic acid to a complementary nucleic acid strand.

Brief Summary Text (57):
Compounds that can be utilized as quenchers of singlet oxygen include, but are not limited to those listed in Table 1. Compounds that can be utilized as quenchers of free radicals include, but are not limited to, those listed in Table 2.

Brief Summary Text (60):
Another embodiment of this invention incorporated water insoluble quenchers into a fluorescent particle conjugate. Such quenchers include but are not limited to quenchers of singlet oxygen, free radicals and excited triplet states; examples of these are given in Tables 1 and 2. A preferred quencher is the carotenoid .beta.-carotene, which can quench singlet oxygen, free radicals and excited triplet states; most other quenchers listed in Tables 1 and 2 possess the identified quenching activities. The water insoluble quenchers in physical contact with the dye molecules interacted efficiently with the reactive species, because they were closer to the source of production of the reactive species than the biological reagent involved in the assay. An example of this embodiment is set forth as Example 8.

Brief Summary Text (62):
Another embodiment of this invention comprised a combination of water insoluble quenchers incorporated into a fluorescent particle conjugated to a biological reagent, in combination with a system which consumes dissolved oxygen present in the solution that contains the conjugate.

Brief Summary Text (63):
Another embodiment of this invention comprised a combination of water soluble quenchers or emulsions of water insoluble quenchers in combination with a system which consumes dissolved oxygen in a solution that contains a fluorescent conjugate.

Brief Summary Text (64):
Another embodiment of this invention comprises a combination of both water insoluble quenchers incorporated into the fluorescent conjugate particle, water soluble quenchers or emulsions of water insoluble quenchers in the solution that contains the conjugate, together with a system which consumes dissolved oxygen in the solution that contains the fluorescent conjugate.

Brief Summary Paragraph Table (1):
TABLE 1 SINGLET OXYGEN QUENCHERS carotenoids: naturally occurring and synthetic tocopherols and tocopheramines: homologues, isomers, derivatives and related compounds thiols: e.g. glutathione, ergothionine, cysteine, N-acetyl- cysteine, dihydrolipoic acid, thiol-containing proteins and peptides) amino acids: tryptophan, tyrosine, histidine, methethione, cysteine, and peptides and proteins containing these amino acids probucol 2,2,6,6-tetra-methyl-piperidine (TEMP) plamitoyl ascorbic acid

caffeine squalene esters of polyunsaturated fatty acids flavonoids lidocaine imidazole and derivatives phthalocyanines and naphthalocyanines p-aminobenzoic acid (PABA) curcumin spermine spermidine merocyanine 540 cholesterol azide

Detailed Description Text (18):
Effect of Dissolved Oxygen Deprivation on the Light Induced Inactivation of Antibodies Coupled to Fluorescent Particles

Detailed Description Text (20):
These results showed that decreasing the level of dissolved oxygen in the incubation mixtures, either by purging the mixture with an inert gas or by including a autoxidizable compound in the mixture, protected the antibody conjugate against light induced inactivation. An advantage of including an antioxidant, such as ascorbate, is particularly evidenced at the longer time point, where ascorbate afforded a greater degree of protection than simply purging the mixture with argon.

Detailed Description Text (24):
These results showed that the addition of an antioxidant and an oxygen consumption system to the storage buffer greatly enhanced the stability of the antibody conjugate to light exposure; here it can be seen that the activity of conjugates incubated in the absence of antioxidants decreased by 85%, whereas conjugates incubated in the presence of an antioxidant and the oxygen consuming system retained their activity.

Detailed Description Text (27):
Particles coupled with anti-troponin I antibody were diluted to 0.14% solids in argon-purged storage buffer prepared with final concentrations of 50 mM sodium ascorbate, 20 mM Trolox (Aldrich Chemical Co., Milwaukee, Wis.), .10 mM sodium azide, and 10 mg/mL glucose. The incubation mixture was contained in two clear, colorless glass vials. Glucose oxidase was added to the mixtures and the vials were argon purged. The vials were then sealed with a septum lined crimp cap. One of the vials was wrapped in foil to exclude light, while the other was left exposed to light. Both vials were placed in a 3-neck round bottom flask, to which a nitrogen gas line, an exhaust line leading to a bubble chamber, and a stopper were attached. The flask was kept under room light and a continuous flow of nitrogen for 3.8 days. Antibody activity was determined as described in Example 1. Results are set forth in Table 8.

Detailed Description Text (28):
These results demonstrated that minimizing or eliminating oxygen from antibody conjugates can prevent the light induced inactivation.

Detailed Description Text (42):
Particles coupled with anti-CKMB antibody were prepared from fluorescent latexes obtained commercially (Molecular Probes, Inc., catalog numbers L7189, L7201 and L7204) and from a different standard fluorescent latex prepared for this and the other experiments herein. Mixtures at 0.14% solids of these particles were prepared by diluting them either into air-saturated storage buffer or into argon-purged storage buffer containing 5 mM sodium ascorbate, 10 mM Trolox, 10 mM sodium azide, 10 mg/mL glucose and 10 .mu.g/mL each catalase and glucose oxidase. Each mixture was split among two clear, colorless glass vials and the vials were sealed with septum lined crimp caps. Vials containing the antioxidant mixtures were evacuated with argon prior to the addition of the glucose oxidase and capping. All vials were placed in a 3-neck round bottom-flask, to which a nitrogen gas line, an exhaust line leading to a bubble chamber, and a stopper were attached. The flask was evacuated with nitrogen, sealed and placed under room light. After a 4 day incubation, samples were withdrawn from the vials and assayed for antibody activity immunoassay.

Detailed Description Text (45):
These results showed that antibody conjugates prepared with a variety of fluorescent dyes were all susceptible to light induced antibody inactivation. It was also seen that, in each case, the inactivation could be decreased by including antioxidants in the storage buffer and minimizing exposure to dissolved oxygen.

Detailed Description Text (55):
Accordingly, the biological reagent in a conjugate was at a maximized distance from the source of radical production at the signal moiety. Maximizing the distance of the biological reagent from the source of the radical production increased the time necessary for diffusion of the radical, and therefore increased the decay of the radical before it reached and then degraded or damaged the biological reagent. Preferred embodiments of the invention comprise conjugates comprising long molecular distances between biological reagent and fluorescent signal moieties, in combination with other inventive embodiments to stabilize the fluorescent conjugates such as an oxygen depleting system, a protein, an antioxidant, or a combination thereof.

Other Reference Publication (10):
I. Rosenthal et al., "Role of Oxygen in the Phototoxicity of Phthalocyanines", Int. J. Radiat. Biol., 67 (1):85-91 (1995).

Other Reference Publication (12):
E. Ullman et al., "Luminescent Oxygen Channeling Assay (LOCI.TM.): Sensitive, Broadly Applicable Homogeneous Immunoassay Method", Clinical Chemistry, 42(9):1518-1526 (1996).

CLAIMS:

1. A method of protecting a biological reagent in a fluorescent conjugate comprising said biological reagent and a fluorescent molecule from inactivation by reactive species generated by the fluorescent molecule on exposure to light and oxygen, the method comprising: associating with said fluorescent conjugate one or more agents that prevent oxidation of said biological reagent by said reactive species generated by said fluorescent molecule, whereby said biological reagent is protected from inactivation by said reactive species, wherein said one or more agent(s) that prevents oxidation of said biological reagent comprises a protein, wherein the protein is juxtaposed between said biological reagent and said fluorescent molecule.

6. A method according to claim 5 wherein said antioxidant is a quencher of a free radical or a quencher of singlet oxygen.

7. A method according to claim 1 wherein said agent that prevents oxidation of said biological reagent comprises an oxygen depleting system.

13. A method according to claim 12 wherein the solution further comprises one or more additional agents that prevent oxidation of said biological reagent, wherein said one or more additional agents are selected from the group consisting of a protein, an antioxidant, and an oxygen depleting system.

15. A method of protecting a biological reagent in a fluorescent conjugate comprising said biological reagent and a fluorescent molecule from inactivation by reactive species generated by the fluorescent molecule on exposure to light and oxygen, the method comprising: dissolving or suspending said fluorescent conjugate in a solution comprising one or more agents that prevent oxidation of said biological reagent by reactive species generated by said fluorescent molecule, whereby the biological reagent is protected from inactivation by said reactive species, wherein said one or more agent(s) that

prevents oxidation of said biological reagent comprises a protein, wherein the protein is juxtaposed between said biological reagent and said fluorescent molecule.

16. A method according to claim 15 wherein said agent that prevents oxidation of said biological reagent is selected from the group consisting of an oxygen depleting system, a protein, and an antioxidant.

18. A method according to claim 17 wherein said additional agent to prevent oxidation of said biological reagent is selected from the group consisting of a heterofunctional polyethylene glycol (PEG) derivative, a long chain form of succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), a long chain form of succinimidyl 3-(2-pyridyldithio)propionate (SPDP), a heterofunctional peptide derivative, an oxygen depleting system, a protein juxtaposed between said biological reagent and said fluorescent molecule, and an antioxidant.

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US006544797B1

(12) **United States Patent**
Buechler et al.

(10) **Patent No.: US 6,544,797 B1**
(45) **Date of Patent: *Apr. 8, 2003**

(54) **COMPOSITIONS AND METHODS FOR INHIBITING LIGHT-INDUCED INACTIVATION OF BIOLOGICAL REAGENTS**

(75) Inventors: **Kenneth F. Buechler**, San Diego, CA (US); **Paul H. McPherson**, Encinitas, CA (US); **Alfred R. Sundquist**, San Diego, CA (US)

(73) Assignee: **Biosite Diagnostics, Inc.**, San Diego, CA (US)

(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/837,309**

(22) Filed: **Apr. 9, 1997**

(51) **Int. Cl.**[7] **C12N 15/09**; C12N 5/10; C12N 15/62

(52) **U.S. Cl.** **436/176**; 436/183; 436/800; 436/546; 436/56; 436/86; 530/402; 435/69.7; 435/172.3; 435/252.3; 435/320.1

(58) **Field of Search** 530/402; 436/176, 436/183, 800, 546, 56, 86; 435/69.7, 172.3, 252.3, 320.1

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E. Ullman et al., "Luminescent Oxygen Channeling Assay (LOCI™): Sensitive, Broadly Applicable Homogeneous Immunoassay Method", *Clinical Chemistry*, 42(9):1518–1526 (1996).

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D. Wohrle, et al., "Phthalocyanines and Related Macrocycles for Multi-electron Transfer in Catalysis, Photochemistry and Photoelectrochemistry", *Polymers for Advanced Technologies*, 6(3):118–130 (1995).

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Assistant Examiner—Lisa V. Daniels-Cook
(74) *Attorney, Agent, or Firm*—Foley & Lardner

(57) **ABSTRACT**

Disclosed is a fluorescent conjugate comprising a biological reagent, a fluorescent molecule, and a means for impeding phototoxic degradation of the biological reagent. The impeding means can be a cross-linking substance having a long molecular distance, whereby the cross-linking substance links the fluorescent molecule and the biological reagent; a quencher of singlet oxygen; a quencher of a free radical; or a combination thereof. Also disclosed is a solution comprising a fluorescent conjugate of a biological reagent and a fluorescent molecule together with an oxygen depleting system.

18 Claims, No Drawings

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L4: Entry 57 of 57 File: USPT Apr 2, 1974

DOCUMENT-IDENTIFIER: US 3801458 A
TITLE: PROCESS FOR PREPARING CEPHALOSPORIN DERIVATIVES

Brief Summary Text (159):
5 ml of clarified filtrate was mixed with 3 ml of 0.2M-sodium pyrophosphate buffer, pH 8.0,1 ml of 10 mM-sodium azide and 1 ml of frozen and thawed cell suspension (224 units of D-amino oxidase activity), and was aerated and incubated at 33.degree. C for 4 hours. Examination of the resultant mixture by thin layer chromatography on silica coated plates developed with 80 percent aqueous acetone showed that 90 percent of the cephalosporin C had been used up and that most of this had been converted to 3-acetoxymethyl-7.beta.-(5-carboxy-5-oxopentanamido) ceph-3-em-4-carboxylic acid and 3-acetoxymethyl-7.beta.-(4-carboxybutanamido)ceph-3-em-4-carboxylic acid.

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DOCUMENT-IDENTIFIER: US 3721607 A
TITLE: REAGENT COMPOSITION AND PROCESS FOR THE DETERMINATION OF GLUCOSE

CLAIMS:

1. Reagent composition for the enzymatic determination of glucose which composition comprises glucose oxidase, peroxidase, a chromogen, a buffer, an azide, and 2,2'-azino-di-(3-ethyl-benzothiazoline-6-sulfonic acid).

12. Method for the enzymatic determination of glucose which method comprises contacting a test sample with a reagent composition comprising glucose oxidase, peroxidase, a chromogen, a buffer, an azide, and 2,2'-azino-di-(3-ethyl-benzothiazoline-6-sulfonic acid).

United States Patent [19]

Gruber et al.

[11] **3,721,607**

[45] **March 20, 1973**

[54] **REAGENT COMPOSITION AND PROCESS FOR THE DETERMINATION OF GLUCOSE**

[75] Inventors: **Wolfgang Gruber**, Garathshausen **Hans Ulrich Bergmeyer**, Tutzing/Obb; **Wolfgang Werner**, Mannheim-Vogelstang; **Erich Bernt**, Munich; **Karlfried Gawehn**, Tutzing/Obb., all of Germany

[73] Assignee: **Boehringer Mannheim GmbH**, Mannheim, Postfach, Germany

[22] Filed: **Aug. 11, 1971**

[21] Appl. No.: **170,949**

[30] **Foreign Application Priority Data**

Aug. 28, 1970 Germany.....P 20 42 828.8

[52] **U.S. Cl.**.....**195/103.5 C**, 195/99

[51] **Int. Cl.**.....**C12k 1/04**

[58] **Field of Search**.....195/103.5 R, 103.5 C

[56] **References Cited**

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Primary Examiner—Alvin E. Tanenholtz
Assistant Examiner—Max D. Hensley
Attorney—Ralph D. Dinklage et al.

[57] **ABSTRACT**

Compositions comprising glucose oxidase, peroxidase, a chromogen, a buffer, an azide, and 2,2'-azino-di-(3-ethylbenzothiazoline-6-sulfonic acid) provide remarkably stable test reagents for the enzymatic determination of glucose.

13 Claims, No Drawings

DOCUMENT-IDENTIFIER: US 4040908 A
TITLE: Polarographic analysis of cholesterol and other macromolecular substances

Brief Summary Text (13):
The method is especially useful for measuring cholesterol in serum with the advantages obtained as described above. Also, in another method form, whole blood samples may be analyzed. For example, where enzyme is added to the sample chamber solution containing whole blood samples, the blood's high catalase activity tends to destroy the hydrogen peroxide as rapidly as it is formed by the oxidase. It has been found, however, that the catalase activity of slightly hemolyzed plasma can be overcome by the use of an agent such as sodium azide. For example, in slightly hemolyzed blood having about 20-200 mg% hemoglobin, azide or similar agent can be added to inhibit catalase activity. It has also been discovered that the azide inhibits the spontaneous decomposition of hydrogen peroxide and by virtue of its bacteriocidal properties, it also preserves buffer which is added to control pH. Therefore, this invention provides a method which makes possible new rapid means for measuring cholesterol in plasma and slightly hemolyzed plasma.

United States Patent [19]

Clark, Jr.

[11] **4,040,908**

[45] **Aug. 9, 1977**

[54] **POLAROGRAPHIC ANALYSIS OF CHOLESTEROL AND OTHER MACROMOLECULAR SUBSTANCES**

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[73] Assignee: **Children's Hospital Medical Center**, Cincinnati, Ohio

[21] Appl. No.: **666,252**

[22] Filed: **Mar. 12, 1976**

[51] **Int. Cl.**[2] **G01N 31/14**

[52] **U.S. Cl.** ..... **195/103.5 R**; 195/63; 195/103.5 C; 204/1 E; 204/195 P

[58] **Field of Search** ..... 195/103.5 R, 103.5 C; 204/1 E, 195 P, 195 B

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Primary Examiner—Alvin E. Tanenholtz

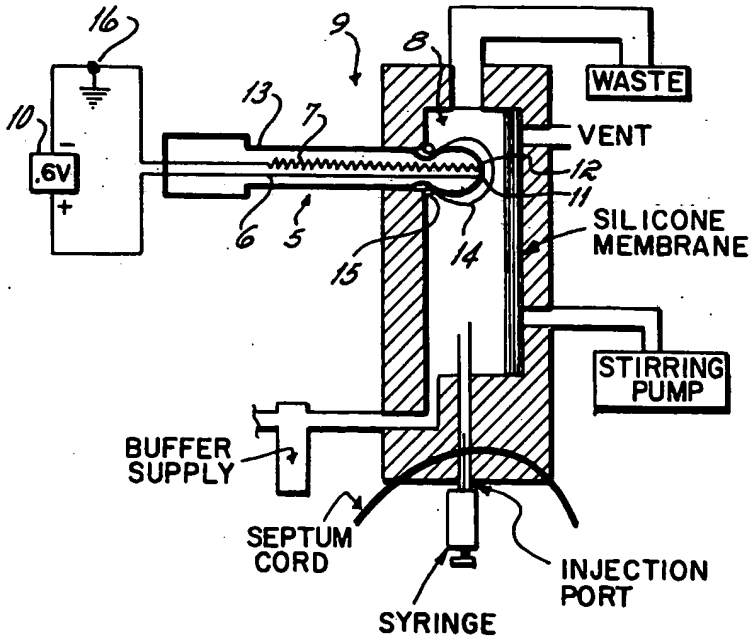
Assistant Examiner—C. A. Fan

Attorney, Agent, or Firm—Wood, Herron & Evans

[57] **ABSTRACT**

A micro-method for measurement of sterols such as cholesterol, and other macromolecular substances, is disclosed utilizing enzymes for the conversion of such substances to produce ultimately hydrogen peroxide and measurement of the generated hydrogen peroxide with a membrane covered polarographic anode. The polarographic anode is set at a voltage so as to produce current proportional to hydrogen peroxide concentration. According to the method of this invention, the macromolecular substance under analysis enters into an enzymatic reaction in a sample chamber on the side of the membrane opposite the anode and the membrane is impermeable to such substances, but senses hydrogen peroxide. The method is adapted to measure free and total blood cholesterol in a precise, rapid, sensitive and specific manner. Other substances of a macromolecular or conventional membrane impermeable nature such as high molecular weight starches or proteins, which undergo enzymatic reaction to produce ultimately hydrogen peroxide, may be analyzed by employing this polarographic technique.

23 Claims, 4 Drawing Figures



DOCUMENT-IDENTIFIER: US 5081015 A
TITLE: Enzyme electrode and method for determination of alcohol content using the same

CLAIMS:

14. A method for determination of alcohol content in a sample by amperometric measurement comprising the steps of immersing an enzyme electrode in a sample solution comprising a buffer solution and sodium azide, said enzyme electrode comprising a conductive base material and an immobilized enzyme membrane or an immobilized enzyme layer, said immobilized enzyme membrane or immobilized enzyme layer comprised of a crosslinked reaction product of an alcohol oxidase solution containing alcohol oxidase, reduced glutathione and a crosslinking agent, and measuring with said enzyme electrode either the amount of oxygen consumed or the amount of hydrogen peroxide produced by the oxidation of alcohol catalyzed by said alcohol oxidase.

18. A method for determination of alcohol content in a sample comprising the steps of immersing an enzyme electrode in a sample solution comprising a buffer solution and sodium azide, said enzyme electrode comprising a conductive base material and an immobilized enzyme membrane or an immobilized enzyme layer, said immobilized enzyme membrane or immobilized enzyme layer comprised of a crosslinked reaction product of an alcohol oxidase solution containing alcohol oxidase, a crosslinking agent and reduced glutathione, and measuring with said enzyme electrode the amount of hydrogen peroxide produced by the oxidation of alcohol catalyzed by the alcohol oxidase, wherein the concentration of sodium azide in the buffer solution is 0.1 .mu.M or more and 1 mM or less.

US005081015A

United States Patent [19]

Hayashi et al.

[11] Patent Number: 5,081,015

[45] Date of Patent: Jan. 14, 1992

[54] ENZYME ELECTRODE AND METHOD FOR DETERMINATION OF ALCOHOL CONTENT USING THE SAME

[75] Inventors: Ryuzo Hayashi, Higashiosaka; Akio Kariyone, Kyoto; Yoshio Hashizume, Kakogawa, all of Japan

[73] Assignee: Kanzaki Paper Mfg. Co., Ltd., Tokyo, Japan

[21] Appl. No.: 401,132

[22] Filed: Aug. 30, 1989

[30] Foreign Application Priority Data

Aug. 30, 1988 [JP] Japan 63-217641
Aug. 31, 1988 [JP] Japan 63-218844

[51] Int. Cl.[5] C12Q 1/54

[52] U.S. Cl. 435/14; 435/177; 435/817; 435/921; 435/938; 204/403

[58] Field of Search 435/817, 25, 34, 190, 435/921, 938, 14, 4, 177; 204/403

[56] References Cited

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Primary Examiner—David L. Lacey
Assistant Examiner—Janelle D. Waack
Attorney, Agent, or Firm—Wenderoth, Lind & Ponack

[57] ABSTRACT

An enzyme electrode possessing an immobilized enzyme membrane or an immobilized enzyme layer composed by applying a solution containing alcohol oxidase and a crosslinking agent, in which the solution further contains reduced glutathione.

According to the invention it is possible to immobilize alcohol oxidase stably, and an excellent immobilized enzyme electrode for alcohol measurement is obtained. Furthermore, by this invention, a highly sensitive and stable measuring method is conducted quite easily.

18 Claims, 4 Drawing Sheets